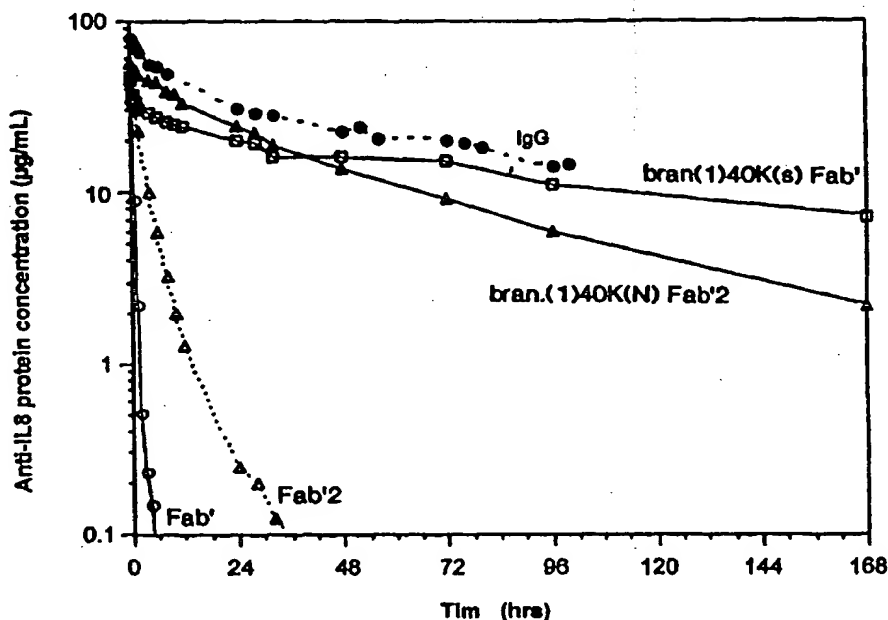




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(54) Title: ANTIBODY FRAGMENT-POLYMER CONJUGATES AND HUMANIZED ANTI-IL-8 MONOCLONAL ANTIBODIES



(57) Abstract

Humanized anti-IL-8 monoclonal antibodies and variants thereof are described for use in diagnostic applications and in the treatment of inflammatory disorders. Also described is a conjugate formed by an antibody fragment covalently attached to a non-proteinaceous polymer, wherein the apparent size of the conjugate is at least about 500 kD. The conjugate exhibits substantially improved half-life, mean residence time, and/or clearance rate in circulation as compared to the underivatized parental antibody fragment.

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ANTIBODY FRAGMENT-POLYMER CONJUGATES AND HUMANIZED ANTI-IL-8 MONOCLONAL ANTIBODIES

FIELD OF THE INVENTION

5 This application relates to the field of antibody fragments derivatized with polymers, and in particular to the use of such derivatization to increase the circulation half-lives of antibody fragment-polymer conjugates. This application also relates to humanized anti-interleukin-8 (IL-8) antibodies and to high affinity variants of such antibodies.

BACKGROUND

10 Modification of proteins with polyethylene glycol ("PEGylation") has the potential to increase residence time and reduce immunogenicity in vivo. For example, Knauf et al., J. Biol. Chem., 263: 15064-15070 (1988) reported a study of the pharmacodynamic behavior in rats of various polyoxylated glycerol and polyethylene glycol modified species of interleukin-2. Despite the known advantage of PEGylation, PEGylated proteins have not been widely exploited for clinical applications. In the case of antibody
15 fragments, PEGylation has not been shown to extend serum half-life to useful levels. Delgado et al., Br. J. Cancer, 73: 175-182 (1996), Kitamura et al., Cancer Res., 51: 4310-4315 (1991), Kitamura et al., Biochem. Biophys. Res. Comm., 171: 1387-1394 (1990), and Pedley et al., Br. J. Cancer, 70: 1126-1130 (1994) reported studies characterizing blood clearance and tissue uptake of certain anti-tumor antigen antibodies r antibody fragments derivatized with low molecular weight (5 kD) PEG. Zapata et al., FASEB J., 9: A1479
20 (1995) reported that low molecular weight (5 or 10 kD) PEG attached to a sulfhydryl group in the hinge region of a Fab' fragment reduced clearance compared to the parental Fab' molecule.

 Interleukin-8 (IL-8) is neutrophil chemotactic peptide secreted by a variety of cells in response to inflammatory mediators (for a review see Hebert *et al.* Cancer Investigation 11(6):743 (1993)). IL-8 can play an important role in the pathogenesis of inflammatory disorders, such as adult respiratory distress
25 syndrome (ARDS), septic shock, and multiple organ failure. Immune therapy for such inflammatory disorders can include treatment of an affected patient with anti-IL-8 antibodies.

 Sticherling *et al.* (J. Immunol. 143:1628 (1989)) disclose the production and characterization of four monoclonal antibodies against IL-8. WO 92/04372, published March 19, 1992, discloses polyclonal antibodies which react with the receptor-interacting site of IL-8 and peptide analogs of IL-8, along with the
30 use of such antibodies to prevent an inflammatory response in patients. St. John *et al.* (Chest 103:932 (1993)) review immune therapy for ARDS, septic shock, and multiple organ failure, including the potential therapeutic use of anti-IL-8 antibodies. Sekido *et al.* (Nature 365:654 (1993)) disclose the prevention of lung reperfusion injury in rabbits by a monoclonal antibody against IL-8. Mulligan *et al.* (J. Immunol. 150:5585 (1993)), disclose protective effects of a murine monoclonal antibody to human IL-8 in
35 inflammatory lung injury in rats.

 WO 95/23865 (International Application No. PCT/US95/02589 published September 8, 1995) demonstrates that anti-IL-8 monoclonal antibodies can be used therapeutically in the treatment of other inflammatory disorders, such as bacterial pneumonias and inflammatory bowel disease.

Anti-IL-8 antibodies are additionally useful as reagents for assaying IL-8. For example, Sticherling *et al.* (Arch. Dermatol. Res. 284:82 (1992)), disclose the use of anti-IL-8 monoclonal antibodies as reagents in immunohistochemical studies. Ko *et al.* (J. Immunol. Methods 149:227 (1992)) disclose the use of anti-IL-8 monoclonal antibodies as reagents in an enzyme-linked immunoabsorbent assay (ELISA) for IL-8.

SUMMARY OF THE INVENTION

One aspect of the invention is a conjugate consisting essentially of one or more antibody fragments covalently attached to one or more polymer molecules, wherein the apparent size of the conjugate is at least about 500 kD.

Another aspect of the invention is an anti-IL-8 monoclonal antibody or antibody fragment comprising the complementarity determining regions of the 6G4.2.5LV11N35E light chain polypeptide amino acid sequence of Fig. 45 (SEQ ID NO:).

Further aspects of the invention are a nucleic acid molecule comprising a nucleic acid sequence encoding the above-described anti-IL-8 monoclonal antibody or antibody fragment; an expression vector comprising the nucleic acid molecule operably linked to control sequences recognized by a host cell transfected with the vector; a host cell transfected with the vector; and a method of producing the antibody fragment comprising culturing the host cell under conditions wherein the nucleic acid encoding the antibody fragment is expressed, thereby producing the antibody fragment, and recovering the antibody fragment from the host cell.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 is a graph depicting the blocking of IL-8 mediated elastase release from neutrophils by anti-IL-8 monoclonal antibody 5.12.14.

Figure 2 is a graph depicting the inhibition of ^{125}I -IL-8 binding to neutrophils by unlabeled IL-8.

Figure 3 demonstrates that a isotype matched negative control Fab (denoted as "4D5 Fab") does not inhibit the binding of ^{125}I -IL-8 to human neutrophils.

Figure 4 is a graph depicting the inhibition of binding of ^{125}I -IL-8 to human neutrophils by chimeric 5.12.14 Fab with an average IC_{50} of 1.6 nM.

Figure 5 is a graph depicting the inhibition of binding of ^{125}I -IL-8 to human neutrophils by chimeric 6G.4.25 Fab with an average IC_{50} of 7.5 nM.

Figure 6 demonstrates the inhibition of human IL-8 mediated neutrophil chemotaxis by chimeric 6G4.2.5 Fab and chimeric 5.12.14 Fab.

Figure 7 demonstrates the relative abilities of chimeric 6G4.2.5 Fab and chimeric 5.12.14 Fab to inhibit rabbit IL-8 mediated neutrophil chemotaxis.

Figure 8 depicts the stimulation of elastase release from human neutrophils by various concentrations of human and rabbit IL-8. The relative extent of elastase release was quantitated by measurement of absorbance at 405 nm. The data represent mean \pm SEM of triplicate samples.

Figure 9 is a graph depicting the ability of chimeric 6G4.2.5 Fab and chimeric 5.12.14 Fab to inhibit elastase release from human neutrophils stimulated by human IL-8. The results were normalized to reflect the percentage of elastase release elicited by 100 nM IL-8 alone. The data represent the mean \pm SEM of three separate experiments performed on different days with different blood donors. IC₅₀ values were calculated by four parameter fit.

Figure 10 is a graph depicting the relative abilities of chimeric 6G4.2.5 Fab and chimeric 5.12.14 Fab to inhibit elastase release from human neutrophils stimulated by rabbit IL-8. The results were normalized to reflect the percentage of elastase release elicited by 100 nM IL-8 alone. The data represent the mean \pm SEM of three separate experiments performed on different days with different blood donors. IC₅₀ values were calculated by four parameter fit.

Figures 11A-11J are a set of graphs depicting the following parameters in a rabbit ulcerative colitis model: Figure 11A depicts myeloperoxidase levels in tissue; Figure 11B depicts IL-8 levels in tissue; Figure 11C depicts colon weight; Figure 11D depicts gross inflammation; Figure 11E depicts edema; Figure 11F depicts extent of necrosis; Figure 11G depicts severity of necrosis; Figure 11H depicts neutrophil margination; Figure 11I depicts neutrophil infiltration; and Figure 11J depicts mononuclear infiltration.

Figure 12 is a graph depicting the effect of anti-IL-8 monoclonal antibody treatment on the number of neutrophils in bronchoalveolar lavage (BAL) fluid in animals infected with Streptococcus pneumoniae, Escherichia coli, or Pseudomonas aeruginosa. Treatment with 6G4.2.5 significantly reduced the number of neutrophils present in the BAL fluid compared to animals treated with isotype control mouse IgG (Figure 12).

Figure 13 depicts the DNA sequences (SEQ ID NOS: 1-6) of three primers designed for each of the light and heavy chains. Multiple primers were designed in order to increase the chances of primer hybridization and efficiency of first strand cDNA synthesis for cloning the variable light and heavy regions of monoclonal antibody 5.12.14.

Figure 14 depicts the DNA sequences (SEQ ID NOS: 7-10) of one forward primer and one reverse primer for the 5.12.14 light chain variable region amplification.

Figure 15 depicts the DNA sequences (SEQ ID NOS: 11-18) of one forward primer and one reverse primer for the 5.12.14 heavy chain variable region amplification.

Figure 16 depicts the DNA sequence (SEQ ID NO: 19) and the amino acid sequence (SEQ ID NO: 20) of the 5.12.14 light chain variable region and partial murine constant light region. CDRs are indicated by either X-ray crystallography (underlined amino acids) or by Kabat sequence comparison (amino acids denoted with asterisk). Important restriction sites are indicated in italics. The signal peptide of STII is amino acids -23 to -1. The murine variable light region is amino acids 1 to 109. The partial murine constant light region is amino acids 110 to 123 (in italics).

Figure 17 depicts the DNA sequence (SEQ ID NO: 21) and the amino acid sequence (SEQ ID NO: 22) of the 5.12.14 heavy chain variable region and partial murine constant heavy region. CDRs are indicated by either X-ray crystallography (underlined amino acids) or by Kabat sequence comparison

(amino acids denoted with asterisk). Important restriction sites are indicated in italics. The signal peptide of STII is amino acids -23 to -1. The murine variable heavy region is amino acids 1 to 120. The partial murine constant heavy region is amino acids 121 to 130.

5 Figure 18 depicts the DNA sequences (SEQ ID NOS: 23-26) of amplification primers used to convert murine light and heavy chain constant region residues to their human equivalents.

Figure 19 depicts the DNA sequence (SEQ ID NO: 27) and the amino acid sequence (SEQ ID NO: 28) for the 5.12.14 light chain variable region and the human IgG1 light chain constant region. CDRs are indicated by either X-ray crystallography (underlined amino acids) or by Kabat sequence comparison (amino acids denoted with asterisk). The human constant region is denoted in italics. The signal peptide of STII is amino acids -23 to -1. The murine variable light region is amino acids 1 to 109. The human constant light region is amino acids 110 to 215.

10 Figures 20A-20B depict the DNA sequence (SEQ ID NO: 29) and the amino acid sequence (SEQ ID NO: 30) for the 5.12.14 heavy chain variable region and the heavy chain constant region of human IgG1. CDRs are indicated by either X-ray crystallography (underlined amino acids) or by Kabat sequence comparison (amino acids denoted with asterisk). The human constant region is denoted in italics. The signal peptide of STII is amino acids -23 to -1. The murine variable heavy region is amino acids 1 to 120. The human constant heavy region is amino acids 121 to 229.

Figure 21 depicts the DNA sequences (SEQ ID NOS: 31-36) of three primers designed for each of the light and heavy chains. Multiple primers were designed in order to increase the chances of primer hybridization and efficiency of first strand cDNA synthesis for cloning the variable light and heavy regions of monoclonal antibody 6G4.2.5.

20 Figure 22 depicts the DNA sequences (SEQ ID NOS: 37-40) of one forward primer and one reverse primer for the 6G4.2.5 light chain variable region amplification.

Figure 23 depicts the DNA sequences (SEQ ID NOS: 41-46) of one forward primer and one reverse primer for the 6G4.2.5 heavy chain variable region amplification.

25 Figure 24 depicts the DNA sequence (SEQ ID NO: 47) and the amino acid sequence (SEQ ID NO: 48) of the 6G4.2.5 light chain variable region and partial murine constant light region. CDRs are indicated by either X-ray crystallography (underlined amino acids) or by Kabat sequence comparison (amino acids denoted with asterisk). Useful cloning sites are in italics. The signal peptide of STII is amino acids -23 to -1. The murine variable light region is amino acids 1 to 114. The partial murine constant light region is amino acids 115 to 131.

30 Figure 25 depicts the DNA sequence (SEQ ID NO: 49) and the amino acid sequence (SEQ ID NO: 50) of the 6G4.2.5 heavy chain variable region and partial murine constant heavy region. CDRs are indicated by either X-ray crystallography (underlined amino acids) or by Kabat sequence comparison (amino acids denoted with asterisk). Useful cloning sites are in italics. The signal peptide of STII is amino acids -23 to -1. The murine variable heavy region is amino acids 1 to 122. The partial murine constant heavy region is amino acids 123 to 135.

Figure 26 depicts the DNA sequences (SEQ ID NOS: 51-54) of primers to convert the murine light chain and heavy chain constant regions to their human equivalents.

Figures 27A-27B depict the DNA sequence (SEQ ID NO: 55) and the amino acid sequence (SEQ ID NO: 56) for the chimeric 6G4.2.5 light chain. CDRs are indicated by either X-ray crystallography (underlined amino acids) or by Kabat sequence comparison (amino acids denoted with asterisk). The human constant region is denoted in italics. The signal peptide of STII is amino acids -23 to -1. The murine variable heavy region is amino acids 1 to 114. The human constant heavy region is amino acids 115 to 220.

Figures 28A-28B depict the DNA sequence (SEQ ID NO: 57) and the amino acid sequence (SEQ ID NO: 58) for the chimeric 6G4.2.5 heavy chain. CDRs are indicated by either X-ray crystallography (underlined amino acids) or by Kabat sequence comparison (amino acids denoted with asterisk). The human constant region is denoted in italics. The signal peptide of STII is amino acids -23 to -1. The murine variable heavy region is amino acids 1 to 122. The human constant heavy region is amino acids 123 to 231.

Fig. 29 depicts an amino acid sequence alignment of murine 6G425 light chain variable domain (SEQ ID NO: 59), humanized 6G425 F(ab)-1 light chain variable domain (SEQ ID NO: 60), and human light chain κI consensus framework (SEQ ID NO: 61) amino acid sequences, and an amino acid sequence alignment of murine 6G425 heavy chain variable domain (SEQ ID NO: 62), humanized 6G425 F(ab)-1 heavy chain variable domain (SEQ ID NO: 63), and human IgG1 subgroup III heavy chain variable domain (SEQ ID NO: 64) amino acid sequences, used in the humanization of 6G425. Light-chain CDRs are labeled L1, L2, L3; heavy chain CDRs are labeled H1, H2, and H3. = and + indicate CDR sequences as defined by X-ray crystallographic contacts and sequence hypervariability, respectively. # indicates a difference between the aligned sequences. Residue numbering is according to Kabat *et al.* Lower case lettering denotes the insertion of an amino acid residue relative to the humIII consensus sequence numbering.

Fig. 30 is a graph with three panels (A, B and C) depicting the ability of F(ab)-9 (humanized 6G4V11 Fab) to inhibit human wild type IL-8, human monomeric IL-8, and rhesus IL-8 mediated neutrophil chemotaxis, respectively. Panel A presents inhibition data for F(ab)-9 samples at concentrations of 0.06 nM, 6.25 nM, 12.5 nM, 25 nM, 50 nM, and 100 nM, for an isotype control antibody (denoted "4D5") sample at a concentration of 100 nM, and for a no antibody control sample, in the presence of 2nM human wild type IL-8. Panel B presents inhibition data for F(ab)-9 samples at concentrations of 6.25 nM, 12.5 nM, 25 nM, and 50 nM, for an isotype control antibody (denoted "4D5") sample at a concentration of 100 nM, and for a no antibody control sample, in the presence of 4 nM human monomeric IL-8 (denoted as "BD59" and as "monomeric IL-8"). Panel C presents inhibition data for F(ab)-9 samples at concentrations of 1 nM, 12.5 nM, 25 nM, and 50 nM, for an isotype control antibody (denoted "4D5") sample at a concentration of 100 nM, and for a no antibody control sample, in the presence of 2 nM rhesus IL-8. In addition, all panels A, B and C each presents data for a no IL-8 buffer control sample (denoted as "Buffer") in the respective inhibition assay.

Fig. 31A depicts the amino acid sequences of the humanized anti-IL-8 6G4.2.5V11-light chain in an N-terminal fusion with the STII leader peptide (SEQ ID NO: 65), the humanized anti-IL-8 6G4.2.5V11

heavy chain in an N-terminal fusion with the STII leader peptide (SEQ ID NO: 66), and a peptide linker in a C-terminal fusion with M13 phage gene-III coat protein (SEQ ID NO: 67).

Fig. 31B depicts the nucleic acid sequence (SEQ ID NO: 68) and the translated amino acid sequence (SEQ ID NO: 65) of the humanized anti-IL-8 6G4.2.5V11 light chain in an N-terminal fusion with the STII leader peptide.

Fig. 31C depicts the amino acid sequences of the humanized anti-IL-8 6G4.2.5V19 light chain in an N-terminal fusion with the STII leader peptide (SEQ ID NO: 69), and the humanized anti-IL-8 6G4.2.5V19 heavy chain in an N-terminal fusion with the STII leader peptide (SEQ ID NO: 70).

Fig. 32 is a three dimensional computer model of the humanized anti-IL-8 6G4.2.5V11 antibody. Heavy chain CDR loops and variable domain regions appear in purple, and CDR-H3 side chain residues appear in yellow. Heavy chain constant domain regions appear in red. Light chain CDR loops and variable domain regions appear in off-white, and the Asn residue at amino acid position 35 (N35) in CDR L1 appears in green. Light chain constant domain regions appear in amber.

Fig. 33 is a Scatchard plot depicting the inhibition of 125 I-IL-8 binding to human neutrophils exhibited by intact murine 6G4.2.5 antibody (denoted 6G4 murine mAb), 6G4.2.5 murine-human chimera Fab (denoted 6G4 chimera), humanized 6G4.2.5 Fab versions I and II (denoted V1 and V11), and variant 6G4.2.5V11N35A Fab (denoted V11N35A).

Fig. 34 is a graph with four panels (A, B, C, and D) depicting the ability of 6G4.2.5V11N35A Fab to inhibit human wild-type IL-8, human monomeric IL-8, rabbit IL-8, and rhesus IL-8 mediated neutrophil chemotaxis, respectively. Panel A presents inhibition data for 6G4.2.5V11N35A Fab samples at concentrations of 0.5, 1, 2, 4, 8, 16, and 33 nM, for an isotype control antibody (denoted "4D5") sample at a concentration of 33 nM, and for a no antibody control (denoted "HuIL-8") sample, in the presence of 2 nM human wild type IL-8. Panel B presents inhibition data for 6G4.2.5V11N35A Fab samples at concentrations of 0.5, 1, 2, 4, 8, 16, and 33 nM, for an intact 6G4.2.5 mAb sample at a concentration of 33 nM, for an isotype control antibody (denoted as "4D5") sample at a concentration of 33 nM, and for a no antibody control (denoted "BD59") sample, in the presence of 2 nM human monomeric IL-8. Panel C presents inhibition data for 6G4.2.5V11N35A Fab samples at concentrations of 0.5, 1, 2, 4, 8, 16, and 33 nM, for an intact 6G4.2.5 mAb sample at a concentration of 33 nM, for an isotype control antibody (denoted "4D5") sample at a concentration of 33 nM, and for a no antibody control (denoted "Rab IL-8") sample, in the presence of 2 nM rabbit IL-8. Panel D presents inhibition data for 6G4.2.5V11N35A Fab samples at concentrations of 0.5, 1, 2, 4, 8, 16, and 33 nM, for an intact 6G4.2.5 mAb sample at a concentration of 33 nM, for an isotype control antibody (denoted as "4D5") sample at a concentration of 33 nM, and for a no antibody control (denoted "Rhe IL-8") sample, in the presence of 2 nM rhesus IL-8. In addition, panels B, C and D each presents data for human wild type IL-8 control (denoted "HuIL-8") samples at a concentration of 2 nM in the respective assay, and panels A, B, C, and D each presents data for a no IL-8 buffer control (denoted "Buffer") sample in the respective assay.

Fig. 35 depicts the amino acid sequences of the humanized anti-IL-8 6G4.2.5V11N35A light chain

in an N-terminal fusion with the STII leader peptide (SEQ ID NO: 71), the humanized anti-IL-8 6G4.2.5V11N35A heavy chain in an N-terminal fusion with the STII leader peptide (SEQ ID NO: 66), and the GCN4 leucine zipper peptide (SEQ ID NO: 72). The Ala residue (substituted for the wild type Asn residue) at amino acid position 35 in the 6G4.2.5V11N35A light chain appears in bold case. A putative pepsin-cleavage site in the GCN4-leucine zipper sequence is underlined.

Fig. 36 depicts the DNA sequence (SEQ ID NO: 73) and the amino acid sequence (SEQ ID NO: 71) of the humanized anti-IL-8 6G4.2.5V11N35A light chain in an N-terminal fusion with the STII leader peptide. Complementarity determining regions L1, L2, and L3 are underlined

Figs. 37A-37B depict the DNA sequence (SEQ ID NO: 74) and the amino acid sequence (SEQ ID NO: 75) of the humanized anti-IL-8 6G4.2.5V11N35A heavy chain in an N-terminal fusion with the STII leader peptide and in a C-terminal fusion with the GCN4 leucine zipper sequence. Complementarity determining regions H1, H2, and H3 are underlined.

Fig. 38 is a Scatchard plot depicting the inhibition of 125 I-IL-8 binding to human neutrophils exhibited by 6G4.2.5V11N35A Fab (denoted Fab), 6G4.2.5V11N35A F(ab')₂ (denoted F(ab')₂), and human wild type IL-8 control (denoted IL-8).

Fig. 39 is a graph depicting a comparison of the wild type human IL-8 mediated neutrophil chemotaxis inhibition activities of the 6G4.2.5V11N35A F(ab')₂ and 6G4.2.5V11N35A Fab. Inhibition data are presented for 6G4.2.5V11N35A Fab samples (denoted "N35A Fab") and 6G4.2.5V11N35A F(ab')₂ samples (denoted N35A F(ab')₂) at concentrations of 0.3, 1, 3, 10, 30, and 100 nM, for an isotype control antibody (denoted as "4D5") sample at a concentration of 100 nM, and for a no antibody control sample, in the presence of 2 nM human wild type IL-8. In addition, inhibition data are presented for no IL-8 buffer control samples (denoted "Buffer").

Fig. 40 is a graph depicting the ability of 6G4.2.5V11N35A F(ab')₂ to inhibit human monomeric IL-8, rhesus IL-8, and rabbit IL-8 mediated neutrophil chemotaxis. Human monomeric IL-8 mediated neutrophil chemotaxis data are presented for 6G4.2.5V11N35A F(ab')₂ samples at concentrations of 0.3, 1, 3, and 10 nM, for an isotype control antibody (denoted as "4D5") sample at a concentration of 100 nM, and for a no antibody control sample (denoted as "BD59"), in the presence of human monomeric IL-8 (denoted as "BD59") at a concentration of 0.5 nM. Rhesus IL-8 mediated neutrophil chemotaxis data are presented for 6G4.2.5V11N35A F(ab')₂ samples at concentrations of 0.3, 1, 3, and 10 nM, and for a no antibody control sample, in the presence of rhesus IL-8 at a concentration of 2 nM. Rabbit IL-8 mediated neutrophil chemotaxis data are presented for 6G4.2.5V11N35A F(ab')₂ samples at concentrations of 0.3, 1, 3, and 10 nM, and for a no antibody control sample, in the presence of rabbit IL-8 at a concentration of 2 nM. In addition, inhibition data are presented for a no IL-8 buffer control sample (denoted as "Buffer") and for a 2 nM human wild type IL-8 (denoted as "HuIL-8").

Figs. 41A-41Q depict the nucleic acid sequence (SEQ ID NO: 76) of the p6G4V11N35A.F(ab')₂ vect r.

Fig. 42 depicts the nucleic acid sequences of the stop template primer (SEQ ID NO:) and the NNS randomization primer (SEQ ID NO:) used for random mutagenesis of amino acid position 35 in variable light chain CDR-L1 of humanized antibody 6G4V11.

Fig. 43A is a table of data describing the frequencies of different phage display clones obtained from the randomization of amino acid position 35 in variable light chain CDR-L1 of humanized antibody 6G4V11.

Fig. 43B contains graphs of displacement curves depicting the inhibition of ¹²⁵I-IL-8 binding to neutrophils exhibited by the 6G4V11N35A, 6G4V11N35D, 6G4V11N35E and 6G4V11N35G Fab's.

Fig. 44 contains a graph depicting the typical kinetics of an anti-IL-8 antibody fragment (6G4V11N35A F(ab')₂) binding to IL-8. Fig. 44 also contains a table of data providing the equilibrium constant for 6G4V11N35A Fab binding to IL-8 (rate constants were not determined "ND"), and the equilibrium and rate constants for 6G4V11N35A F(ab')₂ and 6G4V11N35E Fab binding to IL-8.

Fig. 45 depicts the DNA sequence (SEQ ID NO:) and amino acid sequence (SEQ ID NO:) of the 6G4V11N35E light chain in an N-terminal fusion with the STII leader peptide. Complementarity determining regions L1, L2 and L3 are underlined.

Fig. 46 is a graph depicting the ability of 6G4V11N35E Fab to inhibit human IL-8 (dark columns) and rabbit IL-8 (light columns) mediated neutrophil chemotaxis. Data are presented for 6G4V11N35E Fab samples at concentrations of 0.4, 1.2, 3.7, 11 and 33 nM, and for an isotype control antibody (4D5) sample at a concentration of 100 nM, in the presence of 2 nM human IL-8 or 2 nM rabbit IL-8. In addition, inhibition data are presented for a no IL-8 buffer control sample (denoted "Buffer") and for human and rabbit IL-8 control samples (denoted "IL-8").

Fig. 47 depicts the DNA sequence of the sense (SEQ ID NO:) and anti-sense (SEQ ID NO:) strands of a PvuII-XhoI synthetic nucleotide encoding amino acids Leu4 to Phe29 of the 6G4V11N35A heavy chain.

Figs. 48A-48T depict the DNA sequence (SEQ ID NO:) of plasmid p6G4V11N35A.choSD9.

Fig. 49 contains graphs of displacement curves depicting the inhibition of ¹²⁵I-IL-8 binding to neutrophils exhibited by the full length IgG1 forms of variants 6G4V11N35A and 6G4V11N35E.

Figs. 50A-50B are graphs depicting the ability of full length 6G4V11N35A IgG1 and 6G4V11N35E IgG1 to inhibit human IL-8 (Fig. 50A) and rabbit IL-8 (Fig. 50B) mediated neutrophil chemotaxis.

Fig. 51 contains a graph depicting the typical kinetics of a full length anti-IL8 antibody (6G4V11N35A IgG1) binding to IL-8. Fig. 51 also contains a table of data providing the equilibrium and rate constants for full length murine 6G4.2.5 IgG2a, 6G4V11N35A IgG1 and 6G4V11N35E IgG1 binding to IL-8.

Fig. 52 contains graphs of displacement curves depicting the results of an unlabeled IL-8/¹²⁵I-IL-8 competition radioimmunoassay performed with full length 6G4V11N35A IgG1 and 6G4V11N35E IgG1.

Fig. 53 depicts the DNA sequence (SEQ ID NO:) and amino acid sequence (SEQ ID NO:) of the 6G4V11N35A Fab' heavy chain (6G4V11N35A Fab heavy chain modified to contain a cysteine residue in the hinge region).

Figs. 54A-54C contain graphs of displacement curves depicting the IL-8 binding and IC₅₀'s for PEG-maleimide modified 6G4V11N35A Fab' molecules.

Figs. 55A-55C are graphs depicting the ability of PEG-maleimide modified 6G4V11N35A Fab' molecules to inhibit human IL-8 and rabbit IL-8 mediated neutrophil chemotaxis.

Figs. 56A-56C are graphs depicting the ability of PEG-maleimide modified 6G4V11N35A Fab' molecules to inhibit IL-8 mediated release of β -glucuronidase from neutrophils.

Figs. 57A-57B contain graphs of displacement curves depicting the inhibition of ¹²⁵I-IL-8 binding to neutrophils exhibited by PEG-succinimide modified 6G4V11N35A Fab'₂ molecules.

Figs. 58A-58B are graphs depicting the ability of PEG-succinimide modified 6G4V11N35A F(ab')₂ molecules to inhibit human IL-8 mediated neutrophil chemotaxis.

Figs. 59A-59B are graphs depicting the ability of PEG-succinimide modified 6G4V11N35A F(ab')₂ molecules to inhibit human IL-8 mediated release of β -glucuronidase from neutrophils.

Fig. 60 is a graph depicting the theoretical molecular weight (dotted bars) and effective size (solid bars) of PEG-maleimide modified 6G4V11N35A Fab' molecules as determined by SEC-HPLC.

Fig. 61 is an SDS-PAGE gel depicting the electrophoretic mobility of various PEG-maleimide modified 6G4V11N35A Fab' molecules.

Fig. 62 contains size exclusion chromatograms (SEC-HPLC) depicting the retention times and effective (hydrodynamic) sizes of various PEG-succinimide modified 6G4V11N35A F(ab')₂ molecules.

Fig. 63 is a graph depicting the theoretical molecular weight (open columns), effective size determined by SEC-HPLC (solid columns), and the actual molecular weight determined by SEC-light scattering (shaded columns) for various PEG-succinimide modified 6G4V11N35A F(ab')₂ molecules.

Fig. 64 is an SDS-PAGE gel depicting the electrophoretic mobility of various PEG-succinimide modified 6G4V11N35A F(ab')₂ molecules. From left to right, lane 1 contains unmodified F(ab')₂, lane 2 contains F(ab')₂ coupled to two 40 kD branched PEG-succinimide molecules (denoted "Br(2)-40kD(N)-F(ab')₂"), lane 3 contains F(ab')₂ coupled to one 40 kD branched PEG-succinimide molecule (denoted "Br(1)-40kD(N)-F(ab')₂"), lane 4 contains a mixture of F(ab')₂ coupled to four 20 kD linear PEG-succinimide molecules and F(ab')₂ coupled to five 20 kD linear PEG-succinimide molecules (denoted

"L(4+5)-20kD-(N)-Fab'2"), lane 5 contains $F(ab')_2$ coupled to the 20 kD linear PEG-succinimide molecule (denoted "L(1)-20kD-(N)-Fab'2"), and lane 6 contains molecular weight standards.

Fig. 65 contains graphs comparing the serum concentration vs. time profiles of various PEG-maleimide modified 6G4V11N35A Fab' molecules (upper graph) and various PEG-succinimide modified 6G4V11N35A $F(ab')_2$ molecules (lower graph) in rabbits. In the upper graph, "bran.(1)40K(s)Fab'" denotes 6G4V11N35A Fab' coupled to one 40 kD branched PEG-maleimide molecule, "lin.(1)40K(s)Fab'" denotes 6G4V11N35A Fab' coupled to one 40 kD linear PEG-maleimide molecule, "lin.(1)30K(s)Fab'" denotes 6G4V11N35A Fab' coupled to one 30 kD linear PEG-maleimide molecule, "lin.(1)20K(s)Fab'" denotes 6G4V11N35A Fab' coupled to one 20 kD linear PEG-maleimide molecule. In the lower graph, "bran.(2)40K(N)Fab'2" denotes 6G4V11N35A $F(ab')_2$ coupled to two 40 kD branched PEG-succinimide molecules, "bran.(1)40K(N)Fab'2" denotes 6G4V11N35A $F(ab')_2$ coupled to one 40 kD branched PEG-succinimide molecule, and "Fab'2" denotes unmodified 6G4V11N35A $F(ab')_2$. In both graphs, "IgG" denotes a full length IgG1 equivalent of the human-murine chimeric anti-rabbit IL-8 Fab described in Example F below.

Fig. 66 contains graphs comparing the serum concentration vs. time profiles of 6G4V11N35A Fab' coupled to one 40 kD branched PEG-maleimide molecule (denoted as "bran.(1)40K(s)Fab'"), 6G4V11N35A $F(ab')_2$ coupled to one 40 kD branched PEG-succinimide molecule (denoted as "bran.(1)40K(N)Fab'2"), unmodified 6G4V11N35A $F(ab')_2$ (denoted as "Fab'2"), unmodified 6G4V11N35A Fab' (denoted as "Fab'"), and a full length IgG1 (denoted as "IgG") equivalent of the human-murine chimeric anti-rabbit IL-8 Fab described in Example F below.

Fig. 67 is a graph depicting the effect of 6G4V11N35A Fab' coupled to one 40 kD branched PEG-maleimide molecule (denoted as "PEG 40 Kd") and murine anti-rabbit IL-8 monoclonal antibody 6G4.2.5 (full length IgG2a) (denoted as "6G4.2.5") on gross weight of entire lung in an ARDS rabbit model.

Fig. 68 is a graph depicting the effect of 6G4V11N35A Fab' coupled to one branched 40 kD PEG-maleimide molecule (denoted as "PEG 40 Kd") and murine anti-rabbit IL-8 monoclonal antibody 6G4.2.5 (full length IgG2a) (denoted as "6G4.2.5") on BAL total leukocyte (light columns) and polymorphonuclear cell (dark columns) counts in an ARDS rabbit model. Untreated (no therapeutics) control animal data is denoted as "Control".

Fig. 69 is a graph depicting the effect of 6G4V11N35A Fab' coupled to one branched 40 kD PEG-maleimide molecule (denoted as "PEG 40 Kd") and murine anti-rabbit IL-8 monoclonal antibody 6G4.2.5 (full length IgG2a) (denoted as "6G4.2.5") on PaO_2/FiO_2 ratio at 24 hours-post treatment (light columns) and 48 hours post-treatment (dark columns) in an ARDS rabbit model. Untreated (no therapeutics) control animal data is denoted as "Control".

DESCRIPTION OF THE PREFERRED EMBODIMENTS

I. DEFINITIONS

In general, the following words or phrases have the indicated definition when used in the description, examples, and claims.

"Polymerase chain reaction" or "PCR" refers to a procedure or technique in which minute amounts of a specific piece of nucleic acid, RNA and/or DNA, are amplified as described in U.S. Patent No. 4,683,195 issued 28 July 1987. Generally, sequence information from the ends of the region of interest or beyond needs to be available, such that oligonucleotide primers can be designed; these primers will be identical or similar in sequence to opposite strands of the template to be amplified. The 5' terminal nucleotides of the two primers can coincide with the ends of the amplified material. PCR can be used to amplify specific RNA sequences, specific DNA sequences from total genomic DNA, and cDNA transcribed from total cellular RNA, bacteriophage or plasmid sequences, etc. See generally Mullis *et al.*, Cold Spring Harbor Symp. Quant. Biol. 51:263 (1987); Erlich, ed., PCR Technology (Stockton Press, NY, 1989). As used herein, PCR is considered to be one, but not the only, example of a nucleic acid polymerase reaction method for amplifying a nucleic acid test sample comprising the use of a known nucleic acid as a primer and a nucleic acid polymerase to amplify or generate a specific piece of nucleic acid.

"Antibodies" (Abs) and "immunoglobulins" (Igs) are glycoproteins having the same structural characteristics. While antibodies exhibit binding specificity to a specific antigen, immunoglobulins include both antibodies and other antibody-like molecules which lack antigen specificity. Polypeptides of the latter kind are, for example, produced at low levels by the lymph system and at increased levels by myelomas.

"Native antibodies and immunoglobulins" are usually heterotetrameric glycoproteins of about 150,000 daltons, composed of two identical light (L) chains and two identical heavy (H) chains. Each light chain is linked to a heavy chain by one covalent disulfide bond, while the number of disulfide linkages varies between the heavy chains of different immunoglobulin isotypes. Each heavy and light chain also has regularly spaced intrachain disulfide bridges. Each heavy chain has at one end a variable domain (V_H) followed by a number of constant domains. Each light chain has a variable domain at one end (V_L) and a constant domain at its other end; the constant domain of the light chain is aligned with the first constant domain of the heavy chain, and the light chain variable domain is aligned with the variable domain of the heavy chain. Particular amino acid residues are believed to form an interface between the light- and heavy-chain variable domains (Clothia *et al.*, J. Mol. Biol. 186:651 (1985); Novotny and Haber, Proc. Natl. Acad. Sci. U.S.A. 82:4592 (1985)).

The term "variable" refers to the fact that certain portions of the variable domains differ extensively in sequence among antibodies and are used in the binding and specificity of each particular antibody for its particular antigen. However, the variability is not evenly distributed throughout the variable domains of antibodies. It is concentrated in three segments called complementarity-determining-regions-(CDRs)-or hypervariable regions both in the light-chain and the heavy-chain variable domains. The more highly

conserved portions of variable domains are called the framework (FR). The variable domains of native heavy and light chains each comprise four FR regions, largely adopting a β -sheet configuration, connected by three CDRs, which form loops connecting, and in some cases forming part of, the β -sheet structure. The CDRs in each chain are held together in close proximity by the FR regions and, with the CDRs from the other chain, contribute to the formation of the antigen-binding site of antibodies (see Kabat *et al.*, Sequences of Proteins of Immunological Interest, Fifth Edition, National Institute of Health, Bethesda, MD (1991)). The constant domains are not involved directly in binding an antibody to an antigen, but exhibit various effector functions, such as participation of the antibody in antibody-dependent cellular toxicity.

Papain digestion of antibodies produces two identical antigen-binding fragments, called "Fab" fragments, each with a single antigen-binding site, and a residual "Fc" fragment, whose name reflects its ability to crystallize readily. Pepsin treatment yields an $F(ab')_2$ fragment that has two antigen-combining sites and is still capable of cross-linking antigen.

"Fv" is the minimum antibody fragment which contains a complete antigen-recognition and -binding site. In a two-chain Fv species, this region consists of a dimer of one heavy- and one light-chain variable domain in tight, non-covalent association. In a single-chain Fv species (scFv), one heavy- and one light-chain variable domain can be covalently linked by a flexible peptide linker such that the light and heavy chains can associate in a "dimeric" structure analogous to that in a two-chain Fv species. It is in this configuration that the three CDRs of each variable domain interact to define an antigen-binding site on the surface of the VH-VL dimer. Collectively, the six CDRs confer antigen-binding specificity to the antibody. However, even a single variable domain (or half of an Fv comprising only three CDRs specific for an antigen) has the ability to recognize and bind antigen, although at a lower affinity than the entire binding site. For a review of scFv see Pluckthun, in *The Pharmacology of Monoclonal Antibodies*, vol. 113, Rosenberg and Moore eds., Springer-Verlag, New York, pp. 269-315 (1994).

The Fab fragment also contains the constant domain of the light chain and the first constant domain (CH1) of the heavy chain. Fab' fragments differ from Fab fragments by the addition of a few residues at the carboxy terminus of the heavy chain CH1 domain including one or more cysteines from the antibody hinge region. Fab'-SH is the designation herein for Fab' in which the cysteine residue(s) of the constant domains bear a free thiol group. $F(ab')_2$ antibody fragments originally were produced as pairs of Fab' fragments which have hinge cysteines between them. Other chemical couplings of antibody fragments are also known.

The "light chains" of antibodies (immunoglobulins) from any vertebrate species can be assigned to one of two clearly distinct types, called kappa (κ) and lambda (λ), based on the amino acid sequences of their constant domains.

Depending on the amino acid sequence of the constant domain of their heavy chains, immunoglobulins can be assigned to different classes. There are five major classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM, and several of these can be further divided into subclasses (isotypes), e.g., IgG₁, IgG₂, IgG₃, IgG₄, IgA₁, and IgA₂. The heavy-chain constant domains that correspond to the different

classes of immunoglobulins are called α , δ , ϵ , γ , and μ , respectively. The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known.

The term "antibody" is used in the broadest sense and specifically covers single monoclonal antibodies (including agonist and antagonist antibodies) and antibody compositions with polypeptidic specificity.

"Antibody fragment", and all grammatical variants thereof, as used herein are defined as a portion of an intact antibody comprising the antigen binding site or variable region of the intact antibody, wherein the portion is free of the constant heavy chain domains (i.e. CH2, CH3, and CH4, depending on antibody isotype) of the Fc region of the intact antibody. Examples of antibody fragments include Fab, Fab', Fab'-SH, F(ab')₂, and Fv fragments; diabodies; any antibody fragment that is a polypeptide having a primary structure consisting of one uninterrupted sequence of contiguous amino acid residues (referred to herein as a "single-chain antibody fragment" or "single chain polypeptide"), including without limitation (1) single-chain Fv (scFv) molecules (2) single chain polypeptides containing only one light chain variable domain, or a fragment thereof that contains the three CDRs of the light chain variable domain, without an associated heavy chain moiety and (3) single chain polypeptides containing only one heavy chain variable region, or a fragment thereof containing the three CDRs of the heavy chain variable region, without an associated light chain moiety; and multispecific or multivalent structures formed from antibody fragments. In an antibody fragment comprising one or more heavy chains, the heavy chain(s) can contain any constant domain sequence (e.g. CH1 in the IgG isotype) found in a non-Fc region of an intact antibody, and/or can contain any hinge region sequence found in an intact antibody, and/or can contain a leucine zipper sequence fused to or situated in the hinge region sequence or the constant domain sequence of the heavy chain(s). Suitable leucine zipper sequences include the jun and fos leucine zippers taught by Kostelny et al., J. Immunol., 148: 1547-1553 (1992) and the GCN4 leucine zipper described in the Examples below.

Unless specifically indicated to the contrary, the term "conjugate" as described and claimed herein is defined as a heterogeneous molecule formed by the covalent attachment of one or more antibody fragment(s) to one or more polymer molecule(s), wherein the heterogeneous molecule is water soluble, i.e. soluble in physiological fluids such as blood, and wherein the heterogeneous molecule is free of any structured aggregate. In the context of the foregoing definition, the term "structured aggregate" refers to (1) any aggregate of molecules in aqueous solution having a spheroid or spheroid shell structure, such that the heterogeneous molecule is not in a micelle or other emulsion structure, and is not anchored to a lipid bilayer, vesicle or liposome; and (2) any aggregate of molecules in solid or insolubilized form, such as a chromatography bead matrix, that does not release the heterogeneous molecule into solution upon contact with an aqueous phase. Accordingly, the term "conjugate" as defined herein encompasses the aforementioned heterogeneous molecule in a precipitate, sediment, bioerodible matrix or other solid capable of releasing the heterogeneous molecule into aqueous solution upon hydration of the solid.

Unless specifically indicated to the contrary, the terms "polymer", "polymer molecule", "nonproteinaceous polymer", and "nonproteinaceous polymer molecule" are used interchangeably and are

defined as a molecule formed by covalent linkage of two or more monomers, wherein none of the monomers is contained in the group consisting of alanine (Ala), cysteine (Cys), aspartic acid (Asp), glutamic acid (Glu), phenylalanine (Phe), glycine (Gly), histidine (His), isoleucine (Ile), lysine (Lys), leucine (Leu), methionine (Met), asparagine (Asn), proline (Pro), glutamine (Gln), arginine (Arg), serine (Ser), threonine (Thr), valine (Val), tryptophan (Trp), and tyrosine (Tyr) residues.

The term "monoclonal antibody" (mAb) as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigenic site. Furthermore, in contrast to conventional (polyclonal) antibody preparations which typically include different antibodies directed against different determinants (epitopes), each mAb is directed against a single determinant on the antigen. In addition to their specificity, the monoclonal antibodies are advantageous in that they can be synthesized by hybridoma culture, uncontaminated by other immunoglobulins. The modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present invention may be made by the hybridoma method first described by Kohler *et al.*, *Nature*, 256:495 (1975), or may be made by recombinant DNA methods (see, e.g., U.S. Patent No. 4,816,567 to Cabilly *et al.*). The "monoclonal antibodies" also include clones of antigen-recognition and binding-site containing antibody fragments (Fv clones) isolated from phage antibody libraries using the techniques described in Clackson *et al.*, *Nature*, 352:624-628 (1991) and Marks *et al.*, *J. Mol. Biol.*, 222:581-597 (1991), for example.

The monoclonal antibodies herein include hybrid and recombinant antibodies produced by splicing a variable (including hypervariable) domain of an anti-IL-8 antibody with a constant domain (e.g. "humanized" antibodies), or a light chain with a heavy chain, or a chain from one species with a chain from another species, or fusions with heterologous proteins, regardless of species of origin or immunoglobulin class or subclass designation, as well as antibody fragments (e.g., Fab, F(ab')₂, and Fv), so long as they exhibit the desired biological activity. (See, e.g., U.S. Pat. No. 4,816,567 to Cabilly *et al.*; Mage and Lamoyi, in Monoclonal Antibody Production Techniques and Applications, pp. 79-97 (Marcel Dekker, Inc., New York, 1987).)

The monoclonal antibodies herein specifically include "chimeric" antibodies (immunoglobulins) in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity (Cabilly *et al.*, *supra*; Morrison *et al.*, Proc. Natl. Acad. Sci. U.S.A. 81:6851 (1984)).

"Humanized" forms of non-human (e.g., murine) antibodies are specific chimeric

immunoglobulins, immunoglobulin chains or fragments thereof (such as Fv, Fab, Fab', F(ab')₂, or other antigen-binding subsequences of antibodies) which contain minimal sequence derived from non-human immunoglobulin. For the most part, humanized antibodies are human immunoglobulins (recipient antibody) in which residues from a complementary-determining region (CDR) of the recipient are replaced by residues from a CDR of a non-human species (donor antibody) such as mouse, rat, or rabbit having the desired specificity, affinity, and capacity. In some instances, Fv framework residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, humanized antibodies can comprise residues which are found neither in the recipient antibody nor in the imported CDR or framework sequences. These modifications are made to further refine and maximize antibody performance. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a human immunoglobulin consensus sequence. The humanized antibody optimally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. For further details see Jones *et al.*, Nature 321:522 (1986); Reichmann *et al.*, Nature 332:323 (1988); and Presta, Curr. Op. Struct. Biol. 2:593 (1992).

"Treatment" refers to both therapeutic treatment and prophylactic or preventative measures. Those in need of treatment include those already with the disorder as well as those prone to have the disorder or those in which the disorder is to be prevented.

"Mammal" for purposes of treatment refers to any animal classified as a mammal, including humans, domestic and farm animals, and zoo, sports, or pet animals, such as dogs, horses, cats, cows, etc. Preferably, the mammal herein is human.

As used herein, protein, peptide and polypeptide are used interchangeably to denote an amino acid polymer or a set of two or more interacting or bound amino acid polymers.

As used herein, the term "inflammatory disorders" refers to pathological states resulting in inflammation, typically caused by neutrophil chemotaxis. Examples of such disorders include inflammatory skin diseases including psoriasis; responses associated with inflammatory bowel disease (such as Crohn's disease and ulcerative colitis); ischemic reperfusion; adult respiratory distress syndrome; dermatitis; meningitis; encephalitis; uveitis; autoimmune diseases such as rheumatoid arthritis, Sjorgen's syndrome, vasculitis; diseases involving leukocyte diapedesis; central nervous system (CNS) inflammatory disorder, multiple organ injury syndrome secondary to septicemia or trauma; alcoholic hepatitis, bacterial pneumonia, antigen-antibody complex mediated diseases; inflammations of the lung, including pleurisy, alveolitis, vasculitis, pneumonia, chronic bronchitis, bronchiectasis, and cystic fibrosis; etc. The preferred indications are bacterial pneumonia and inflammatory bowel disease such as ulcerative colitis.

The terms "hydrodynamic size", "apparent size", "apparent molecular weight", "effective size" and "effective molecular weight" of a molecule are used synonymously herein refer to the size of a molecule as determined by comparison to a standard curve produced with globular protein molecular weight standards in a size exclusion chromatography system, wherein the standard curve is created by mapping the actual

molecular weight of each standard against its elution time observed in the size exclusion chromatography system. Thus, the apparent size of a test molecule is derived by using the molecule's elution time to extrapolate a putative molecular weight from the standard curve. Preferably, the molecular weight standards used to create the standard curve are selected such that the apparent size of the test molecule falls within the linear portion of the standard curve.

II. MODES FOR CARRYING OUT THE INVENTION

In one part, the invention arises from the surprising and unexpected discovery that antibody fragment-polymer conjugates having an effective or apparent size significantly greater than the antibody fragment-polymer conjugates described in the art confers an increase in serum half-life, an increase in mean residence time in circulation (MRT), and/or a decrease in serum clearance rate over underivatized antibody fragment which far exceed the modest changes in such biological property or properties obtained with the art-known antibody fragment-polymer conjugates. The present inventors have determined for the first time that increasing the effective size of an antibody fragment to at least about 500,000 D, or increasing the effective size of an antibody fragment by at least about 8 fold over the effective size of the parental antibody fragment, or derivatizing an antibody fragment with a polymer of at least about 20,000 D in molecular weight, yields a molecule with a commercially useful pharmacokinetic profile. The greatly extended serum half-life, extended MRT, and/or reduced serum clearance rate of the conjugates of the invention makes such conjugates viable alternatives to intact antibodies used for therapeutic treatment of many disease indications. Antibody fragments provide significant advantages over intact antibodies, notably the fact that recombinant antibody fragments can be made in bacterial cell expression systems. Bacterial cell expression systems provide several advantages over mammalian cell expression systems, including reduced time and cost at both the research and development and manufacturing stages of a product.

In another part, the present invention also arises from the humanization of the 6G4.2.5 murine anti-rabbit IL-8 monoclonal antibody ("6G4.2.5") described in WO 95/23865 (PCT/US95/02589 published September 8, 1995), the entire disclosure of which is specifically incorporated herein by reference. The hybridoma producing antibody 6G4.2.5 was deposited on September 28, 1994 with the American Type Culture Collection and assigned ATCC Accession No. HB 11722 as described in the Examples below. In one aspect, the invention provides a humanized derivative of the 6G4.2.5 antibody, variant 11 (referred to herein as "6G4.2.5v11"), in which the murine CDRs of 6G4.2.5 are grafted onto a consensus framework for human light chain κ I and human IgG1 heavy chain subgroup III, followed by importing three framework residues from the murine 6G4.2.5 parent heavy chain variable domain sequence into analogous sites in the heavy chain variable domain of the human template sequence, as described in the Examples below. In another aspect, the invention provides variants of the 6G4.2.5v11 antibody with certain amino acid substitution(s) yielding increased affinity for human IL-8 and/or promoting greater efficiency in recombinant manufacturing processes.

It will be understood that in the context of this Section (II) and all subsections thereof, every reference to "an antibody fragment" or "the antibody fragment" contained in a conjugate shall be a reference

to one or more antibody fragment(s) in the conjugate (consistent with the definition of the term "conjugate" set forth in Section (I) above), except where the number of antibody fragment(s) in the conjugate is expressly indicated. It will be understood that in the context of this Section (II) and all subsections thereof, every reference to "a polymer", "a polymer molecule", "the polymer", or "the polymer molecule" contained in a conjugate shall be a reference to one or more polymer molecule(s) in the conjugate (consistent with the definition of the term "conjugate" set forth in Section (I) above), except where the number of polymer molecule(s) in the conjugate is expressly indicated.

1. LARGE EFFECTIVE SIZE ANTIBODY FRAGMENT-POLYMER CONJUGATES

In one aspect, the invention provides an antibody fragment covalently attached to a polymer to form a conjugate having an effective or apparent size of at least about 500,000 Daltons (D). In another aspect, the invention provides an antibody fragment covalently attached to a polymer to form a conjugate having an apparent size that is at least about 8 fold greater than the apparent size of the parental antibody fragment. In yet another aspect, the invention provides an antibody fragment covalently attached to a polymer of at least about 20,000 D in molecular weight (MW). It will be appreciated that the unexpectedly and surprisingly large increase in antibody fragment serum half-life, increase in MRT, and/or decrease in serum clearance rate can be achieved by using any type of polymer or number of polymer molecules which will provide the conjugate with an effective size of at least about 500,000 D, or by using any type of polymer or number of polymer molecules which will provide the conjugate with an effective size that is at least about 8 fold greater than the effective size of the parental antibody fragment, or by using any type or number of polymers wherein each polymer molecule is at least about 20,000 D in MW. Thus, the invention is not dependent on the use of any particular polymer or molar ratio of polymer to antibody fragment in the conjugate.

In addition, the beneficial aspects of the invention extend to antibody fragments without regard to antigen specificity. Although variations from antibody to antibody are to be expected, the antigen specificity of a given antibody will not substantially impair the extraordinary improvement in serum half-life, MRT, and/or serum clearance rate for antibody fragments thereof that can be obtained by derivatizing the antibody fragments as taught herein.

In one embodiment, the conjugate has an effective size of at least about 500,000 D, or at least about 800,000 D, or at least about 900,000 D, or at least about 1,000,000 D, or at least about 1,200,000 D, or at least about 1,400,000 D, or at least about 1,500,000 D, or at least about 1,800,000 D, or at least about 2,000,000 D, or at least about 2,500,000 D.

In another embodiment, the conjugate has an effective size of at or about 500,000 D to at or about 10,000,000 D, or an effective size of at or about 500,000 D to at or about 8,000,000 D, or an effective size of at or about 500,000 D to at or about 5,000,000 D, or an effective size of at or about 500,000 D to at or about 4,000,000 D, or an effective size of at or about 500,000 D to at or about 3,000,000 D, or an effective size of at or about 500,000 D to at or about 2,500,000 D, or an effective size of at or about 500,000 D to at or about 2,000,000 D, or an effective size of at or about 500,000 D to at or about 1,800,000 D, or an

effective size of at or about 500,000 D to at or about 1,600,000 D, or an effective size of at or about 500,000 D to at or about 1,500,000 D, or an effective size of at or about 500,000 D to at or about 1,000,000 D.

5 In another embodiment, the conjugate has an effective size of at or about 800,000 D to at or about 10,000,000 D, or an effective size of at or about 800,000 D to at or about 8,000,000 D, or an effective size of at or about 800,000 D to at or about 5,000,000 D, or an effective size of at or about 800,000 D to at or about 4,000,000 D, or an effective size of at or about 800,000 D to at or about 3,000,000 D, or an effective size of at or about 800,000 D to at or about 2,500,000 D, or an effective size of at or about 800,000 D to at or about 2,000,000 D, or an effective size of at or about 800,000 D to at or about 1,800,000 D, or an effective size of at or about 800,000 D to at or about 1,600,000 D, or an effective size of at or about 800,000 D to at or about 1,500,000 D, or an effective size of at or about 800,000 D to at or about 1,000,000 D.

10 In another embodiment, the conjugate has an effective size of at or about 900,000 D to at or about 10,000,000 D, or an effective size of at or about 900,000 D to at or about 8,000,000 D, or an effective size of at or about 900,000 D to at or about 5,000,000 D, or an effective size of at or about 900,000 D to at or about 4,000,000 D, or an effective size of at or about 900,000 D to at or about 3,000,000 D, or an effective size of at or about 900,000 D to at or about 2,500,000 D, or an effective size of at or about 900,000 D to at or about 2,000,000 D, or an effective size of at or about 900,000 D to at or about 1,800,000 D, or an effective size of at or about 900,000 D to at or about 1,600,000 D, or an effective size of at or about 900,000 D to at or about 1,500,000 D.

20 In another embodiment, the conjugate has an effective size of at or about 1,000,000 D to at or about 10,000,000 D, or an effective size of at or about 1,000,000 D to at or about 8,000,000 D, or an effective size of at or about 1,000,000 D to at or about 5,000,000 D, or an effective size of at or about 1,000,000 D to at or about 4,000,000 D, or an effective size of at or about 1,000,000 D to at or about 3,000,000 D, or an effective size of at or about 1,000,000 D to at or about 2,500,000 D, or an effective size of at or about 1,000,000 D to at or about 2,000,000 D, or an effective size of at or about 1,000,000 D to at or about 1,800,000 D, or an effective size of at or about 1,000,000 D to at or about 1,600,000 D, or an effective size of at or about 1,000,000 D to at or about 1,500,000 D.

30 In a further embodiment, the conjugate has an effective size that is at least about 8 fold greater, or at least about 10 fold greater, or at least about 12 fold greater, or at least about 15 fold greater, or at least about 18 fold greater, or at least about 20 fold greater, or at least about 25 fold greater, or at least about 28 fold greater, or at least about 30 fold greater, or at least about 40 fold greater, than the effective size of the parental antibody fragment.

35 In another embodiment, the conjugate has an effective size that is about 8 fold to about 100 fold greater, or is about 8 fold to about 80 fold greater, or is about 8 fold to about 50 fold greater, or is about 8 fold to about 40 fold greater, or is about 8 fold to about 30 fold greater, or is about 8 fold to about 28 fold greater, or is about 8 fold to about 25 fold greater, or is about 8 fold to about 20 fold greater, or is about 8 fold to about 18 fold greater, or is about 8 fold to about 15 fold greater, than the effective size of the parental antibody fragment.

In another embodiment, the conjugate has an effective size that is about 12 fold to about 100 fold greater, or is about 12 fold to about 80 fold greater, or is about 12 fold to about 50 fold greater, or is about 12 fold to about 40 fold greater, or is about 12 fold to about 30 fold greater, or is about 12 fold to about 28 fold greater, or is about 12 fold to about 25 fold greater, or is about 12 fold to about 20 fold greater, or is about 12 fold to about 18 fold greater, or is about 12 fold to about 15 fold greater, than the effective size of the parental antibody fragment.

In another embodiment, the conjugate has an effective size that is about 15 fold to about 100 fold greater, or is about 15 fold to about 80 fold greater, or is about 15 fold to about 50 fold greater, or is about 15 fold to about 40 fold greater, or is about 15 fold to about 30 fold greater, or is about 15 fold to about 28 fold greater, or is about 15 fold to about 25 fold greater, or is about 15 fold to about 20 fold greater, or is about 15 fold to about 18 fold greater, than the effective size of the parental antibody fragment.

In another embodiment, the conjugate has an effective size that is about 18 fold to about 100 fold greater, or is about 18 fold to about 80 fold greater, or is about 18 fold to about 50 fold greater, or is about 18 fold to about 40 fold greater, or is about 18 fold to about 30 fold greater, or is about 18 fold to about 28 fold greater, or is about 18 fold to about 25 fold greater, or is about 18 fold to about 20 fold greater, than the effective size of the parental antibody fragment.

In another embodiment, the conjugate has an effective size that is about 20 fold to about 100 fold greater, or is about 20 fold to about 80 fold greater, or is about 20 fold to about 50 fold greater, or is about 20 fold to about 40 fold greater, or is about 20 fold to about 30 fold greater, or is about 20 fold to about 28 fold greater, or is about 20 fold to about 25 fold greater, than the effective size of the parental antibody fragment.

In another embodiment, the conjugate has an effective size that is about 25 fold to about 100 fold greater, or is about 25 fold to about 80 fold greater, or is about 25 fold to about 50 fold greater, or is about 25 fold to about 40 fold greater, or is about 25 fold to about 30 fold greater, or is about 25 fold to about 28 fold greater, than the effective size of the parental antibody fragment.

In another embodiment, the conjugate has an effective size that is about 28 fold to about 100 fold greater, or is about 28 fold to about 80 fold greater, or is about 28 fold to about 50 fold greater, or is about 28 fold to about 40 fold greater, or is about 28 fold to about 30 fold greater, than the effective size of the parental antibody fragment.

In another embodiment, the conjugate has an effective size that is about 30 fold to about 100 fold greater, or is about 30 fold to about 80 fold greater, or is about 30 fold to about 50 fold greater, or is about 30 fold to about 40 fold greater, than the effective size of the parental antibody fragment.

In another embodiment, the conjugate has an effective size that is about 40 fold to about 100 fold greater, or is about 40 fold to about 80 fold greater, or is about 40 fold to about 50 fold greater, than the effective size of the parental antibody fragment.

In still another embodiment, the conjugate is an antibody fragment covalently attached to at least one polymer having an actual MW of at least about 20,000 D.

In a further embodiment, the conjugate is an antibody fragment covalently attached to at least one polymer having an actual MW of at least about 30,000 D.

In yet another embodiment, the conjugate is an antibody fragment covalently attached to at least one polymer having an actual MW of at least about 40,000 D.

5 In another embodiment, the conjugate is an antibody fragment covalently attached to at least one polymer having an actual MW that is at or about 20,000 D to at or about 300,000 D, or is at or about 30,000 D to at or about 300,000 D, or is at or about 40,000 D to at or about 300,000 D.

In another embodiment, the conjugate is an antibody fragment covalently attached to at least one polymer having an actual MW that is at or about 20,000 D to at or about 100,000 D, or is at or about 30,000
10 D to at or about 100,000 D, or is at or about 40,000 D to at or about 100,000 D.

In another embodiment, the conjugate is an antibody fragment covalently attached to at least one polymer having an actual MW that is at or about 20,000 D to at or about 70,000 D, or is at or about 30,000 D to at or about 70,000 D, or is at or about 40,000 D to at or about 70,000 D.

In another embodiment, the conjugate is an antibody fragment covalently attached to at least one polymer having an actual MW that is at or about 20,000 D to at or about 50,000 D, or is at or about 30,000
15 D to at or about 50,000 D, or is at or about 40,000 D to at or about 50,000 D.

In another embodiment, the conjugate is an antibody fragment covalently attached to at least one polymer having an actual MW that is at or about 20,000 D to at or about 40,000 D, or is at or about 30,000 D to at or about 40,000 D.

20 The conjugates of the invention can be made using any suitable technique now known or hereafter developed for derivatizing antibody fragments with polymers. It will be appreciated that the invention is not limited to conjugates utilizing any particular type of linkage between an antibody fragment and a polymer.

The conjugates of the invention include species wherein a polymer is covalently attached to a non-specific site or non-specific sites on the parental antibody fragment, i.e. polymer attachment is not targeted
25 to a particular region or a particular amino acid residue in the parental antibody fragment. In such embodiments, the coupling chemistry can, for example, utilize the free epsilon amino groups of lysine residues in the parental antibody as attachment sites for the polymer, wherein such lysine residue amino groups are randomly derivatized with polymer.

In addition, the conjugates of the invention include species wherein a polymer is covalently
30 attached to a specific site or specific sites on the parental antibody fragment, i.e. polymer attachment is targeted to a particular region or a particular amino acid residue or residues in the parental antibody fragment. In such embodiments, the coupling chemistry can, for example, utilize the free sulfhydryl group of a cysteine residue not in a disulfide bridge in the parental antibody fragment. In one embodiment, one or more cysteine residue(s) is (are) engineered into a selected site or sites in the parental antibody fragment for
35 the purpose of providing a specific attachment site or sites for polymer. The polymer can be activated with any functional group that is capable of reacting specifically with the free sulfhydryl or thiol group(s) on the parental antibody, such as maleimide, sulfhydryl, thiol, triflate, tesylate, aziridine, epoxide, and 5-pyridyl

functional groups. The polymer can be coupled to the parental antibody fragment using any protocol suitable for the chemistry of the coupling system selected, such as the protocols and systems described in Section (II)(1)(b) or in Section (T) of the Examples below.

5 In another embodiment, polymer attachment is targeted to the hinge region of the parental antibody fragment. The location of the hinge region varies according to the isotype of the parental antibody. Typically, the hinge region of IgG, IgD and IgA isotype heavy chains is contained in a proline rich peptide sequence extending between the C_H1 and C_H2 domains. In a preferred embodiment, a cysteine residue or residues is (are) engineered into the hinge region of the parental antibody fragment in order to couple polymer specifically to a selected location in the hinge region.

10 In one aspect, the invention encompasses a conjugate having any molar ratio of polymer to antibody fragment that endows the conjugate with an apparent size in the desired range as taught herein. The apparent size of the conjugate will depend in part upon the size and shape of the polymer used, the size and shape of the antibody fragment used, the number of polymer molecules attached to the antibody fragment, and the location of such attachment site(s) on the antibody fragment. These parameters can easily
15 be identified and maximized to obtain the a conjugate with the desired apparent size for any type of antibody fragment, polymer and linkage system.

In another aspect, the invention encompasses a conjugate with a polymer to antibody fragment molar ratio of no more than about 10:1, or no more than about 5:1, or no more than about 4:1, or no more than about 3:1, or no more than about 2:1, or no more than 1:1.

20 In yet another aspect, the invention encompasses a conjugate wherein the antibody fragment is attached to about 10 or fewer polymer molecules, each polymer molecule having a molecular weight of at least about 20,000 D, or at least about 30,000 D, or at least about 40,000 D. In another embodiment, the conjugate contains an antibody fragment attached to about 5 or fewer polymer molecules, each polymer molecule having a molecular weight of at least about 20,000 D, or at least about 30,000 D, or at least about
25 40,000 D. In still another embodiment, the conjugate contains an antibody fragment attached to about 4 or fewer polymer molecules, each polymer molecule having a molecular weight of at least about 20,000 D, or at least about 30,000 D, or at least about 40,000 D. In a further embodiment, the conjugate contains an antibody fragment attached to about 3 or fewer polymer molecules, each polymer molecule having a molecular weight of at least about 20,000 D, or at least about 30,000 D, or at least about 40,000 D. In an
30 additional embodiment, the conjugate contains an antibody fragment attached to about 2 or fewer polymer molecules, each polymer molecule having a molecular weight of at least about 20,000 D, or at least about 30,000 D, or at least about 40,000 D. Also provided herein is a conjugate containing an antibody fragment attached to a single polymer molecule having a molecular weight of at least about 20,000 D, or at least about 30,000 D, or at least about 40,000 D.

35 In still another aspect, the invention encompasses a conjugate wherein every polymer molecule in the conjugate has a molecular weight that is at or about 20,000 D to at or about 300,000 D, or is at or about 30,000 D to at or about 300,000 D, or is at or about 40,000 D to at or about 300,000 D, and wherein the

conjugate contains no more than about 10 polymer molecules, or no more than about 5 polymer molecules, or no more than about 4 polymer molecules, or no more than about 3 polymer molecules, or no more than about 2 polymer molecules, or no more than 1 polymer molecule.

5 In still another aspect, the invention encompasses a conjugate wherein every polymer molecule in the conjugate has a molecular weight that is at or about 20,000 D to at or about 100,000 D, or is at or about 30,000 D to at or about 100,000 D, or is at or about 40,000 D to at or about 100,000 D, and wherein the conjugate contains no more than about 10 polymer molecules, or no more than about 5 polymer molecules, or no more than about 4 polymer molecules, or no more than about 3 polymer molecules, or no more than about 2 polymer molecules, or no more than 1 polymer molecule.

10 In still another aspect, the invention encompasses a conjugate wherein every polymer molecule in the conjugate has a molecular weight that is at or about 20,000 D to at or about 70,000 D, or is at or about 30,000 D to at or about 70,000 D, or is at or about 40,000 D to at or about 70,000 D, and wherein the conjugate contains no more than about 10 polymer molecules, or no more than about 5 polymer molecules, or no more than about 4 polymer molecules, or no more than about 3 polymer molecules, or no more than about 2 polymer molecules, or no more than 1 polymer molecule.

15 In still another aspect, the invention encompasses a conjugate wherein every polymer molecule in the conjugate has a molecular weight that is at or about 20,000 D to at or about 50,000 D, or is at or about 30,000 D to at or about 50,000 D, or is at or about 40,000 D to at or about 50,000 D, and wherein the conjugate contains no more than about 10 polymer molecules, or no more than about 5 polymer molecules, or no more than about 4 polymer molecules, or no more than about 3 polymer molecules, or no more than about 2 polymer molecules, or no more than 1 polymer molecule.

20 In still another aspect, the invention encompasses a conjugate wherein every polymer molecule in the conjugate has a molecular weight that is at or about 20,000 D to at or about 40,000 D, or is at or about 30,000 D to at or about 40,000 D, and wherein the conjugate contains no more than about 10 polymer molecules, or no more than about 5 polymer molecules, or no more than about 4 polymer molecules, or no more than about 3 polymer molecules, or no more than about 2 polymer molecules, or no more than 1 polymer molecule.

25 It is believed that the serum half-life, MRT and/or serum clearance rate of any antibody fragment can be greatly improved by derivatizing the antibody fragment with polymer as taught herein. In one embodiment, the conjugate contains an antibody fragment selected from the group consisting of Fab, Fab', Fab'-SH, Fv, scFv and F(ab')₂.

30 In a preferred embodiment, the conjugate contains an antibody fragment selected from the group consisting of Fab, Fab', and Fab'-SH, wherein every polymer molecule in the conjugate is attached to the hinge region of the antibody fragment.

35 In another preferred embodiment, the conjugate contains an antibody fragment selected from the group consisting of Fab, Fab', and Fab'-SH, every polymer molecule in the conjugate molecule is attached to the hinge region of the antibody fragment, and the conjugate contains no more than about 10 polymer

molecules, or no more than about 5 polymer molecules, or no more than about 4 polymer molecules, or no more than about 3 polymer molecules, or no more than about 2 polymer molecules, or no more than 1 polymer molecule.

In yet another preferred embodiment, the conjugate contains a $F(ab')_2$ antibody fragment attached to no more than about 2 polymer molecules, wherein every polymer molecule is attached to a cysteine residue in the light or heavy chain of the antibody fragment that would ordinarily form the disulfide bridge linking the light and heavy chains, wherein the disulfide bridge is avoided by substituting another amino acid, such as serine, for the corresponding cysteine residue in the opposite chain.

In a further embodiment, the conjugate contains an antibody fragment selected from the group consisting of Fab, Fab', and Fab'-SH, wherein the antibody fragment is attached to no more than 1 polymer molecule and the polymer is coupled to a cysteine residue in the light or heavy chain of the antibody fragment that would ordinarily form the disulfide bridge linking the light and heavy chains, wherein the disulfide bridge is avoided by substituting another amino acid, such as serine, for the corresponding cysteine residue in the opposite chain.

In an additional embodiment, the conjugate contains an antibody fragment selected from the group consisting of Fab, Fab', and Fab'-SH, every polymer molecule in the conjugate is at least about 20,000 D in molecular weight, or at least about 30,000 in molecular weight, or at least about 40,000 D in molecular weight; every polymer molecule in the conjugate is attached to the hinge region of the antibody fragment, and the conjugate contains no more than about 10 polymer molecules, or no more than about 5 polymer molecules, or no more than about 4 polymer molecules, or no more than about 3 polymer molecules, or no more than about 2 polymer molecules, or no more than 1 polymer molecule.

In another embodiment, the conjugate contains an antibody fragment selected from the group consisting of Fab, Fab', and Fab'-SH, every polymer molecule in the conjugate is at or about 20,000 D to at or about 300,000 D in molecular weight, or is at or about 30,000 D to at or about 300,000 D in molecular weight, or is at or about 40,000 D to at or about 300,000 D in molecular weight, every polymer molecule in the conjugate is attached to the hinge region of the antibody fragment, and the conjugate contains no more than about 10 polymer molecules, or no more than about 5 polymer molecules, or no more than about 4 polymer molecules, or no more than about 3 polymer molecules, or no more than about 2 polymer molecules, or no more than 1 polymer molecule.

In another embodiment, the conjugate contains an antibody fragment selected from the group consisting of Fab, Fab', and Fab'-SH, every polymer molecule in the conjugate is at or about 20,000 D to at or about 100,000 D in molecular weight, or is at or about 30,000 D to at or about 100,000 D in molecular weight, or is at or about 40,000 D to at or about 100,000 D in molecular weight, every polymer molecule in the conjugate is attached to the hinge region of the antibody fragment, and the conjugate contains no more than about 10 polymer molecules, or no more than about 5 polymer molecules, or no more than about 4 polymer molecules, or no more than about 3 polymer molecules, or no more than about 2 polymer molecules, or no more than 1 polymer molecule.

In another embodiment, the conjugate contains an antibody fragment selected from the group consisting of Fab, Fab', and Fab'-SH, every polymer molecule in the conjugate is at or about 20,000 D to at or about 70,000 D in molecular weight, or is at or about 30,000 D to at or about 70,000 D in molecular weight, every polymer molecule in the conjugate is attached to the hinge region of the antibody fragment, and the conjugate contains no more than about 10 polymer molecules, or no more than about 5 polymer molecules, or no more than about 4 polymer molecules, or no more than about 3 polymer molecules, or no more than about 2 polymer molecules, or no more than 1 polymer molecule.

In another embodiment, the conjugate contains an antibody fragment selected from the group consisting of Fab, Fab', and Fab'-SH, every polymer molecule in the conjugate is at or about 20,000 D to at or about 50,000 D in molecular weight, or is at or about 30,000 D to at or about 50,000 D in molecular weight, every polymer molecule in the conjugate is attached to the hinge region of the antibody fragment, and the conjugate contains no more than about 10 polymer molecules, or no more than about 5 polymer molecules, or no more than about 4 polymer molecules, or no more than about 3 polymer molecules, or no more than about 2 polymer molecules, or no more than 1 polymer molecule.

In another embodiment, the conjugate contains an antibody fragment selected from the group consisting of Fab, Fab', and Fab'-SH, every polymer molecule in the conjugate is at or about 20,000 D to at or about 40,000 D in molecular weight, or is at or about 30,000 D to at or about 40,000 D in molecular weight, every polymer molecule in the conjugate is attached to the hinge region of the antibody fragment, and the conjugate contains no more than about 10 polymer molecules, or no more than about 5 polymer molecules, or no more than about 4 polymer molecules, or no more than about 3 polymer molecules, or no more than about 2 polymer molecules, or no more than 1 polymer molecule.

In a further embodiment, the conjugate contains a $F(ab')_2$ antibody fragment attached to no more than about 2 polymer molecules, wherein every polymer molecule in the conjugate is at least about 20,000 D in molecular weight, or at least about 30,000 D in molecular weight, or at least about 40,000 D in molecular weight, and wherein every polymer molecule in the conjugate is attached to a cysteine residue in the light or heavy chain of the antibody fragment that would ordinarily form the disulfide bridge linking the light and heavy chains, wherein the disulfide bridge is avoided by substituting another amino acid, such as serine, for the corresponding cysteine residue in the opposite chain.

In another embodiment, the conjugate contains a $F(ab')_2$ antibody fragment attached to no more than about 2 polymer molecules, wherein every polymer molecule in the conjugate is at or about 20,000 D to at or about 300,000 D in molecular weight, or is at or about 30,000 D to at or about 300,000 D in molecular weight, or is at or about 40,000 D to at or about 300,000 D in molecular weight, and wherein every polymer molecule in the conjugate is attached to a cysteine residue in the light or heavy chain of the antibody fragment that would ordinarily form the disulfide bridge linking the light and heavy chains, wherein the disulfide bridge is avoided by substituting another amino acid, such as serine, for the

corresponding cysteine residue in the opposite chain.

In another embodiment, the conjugate contains a $F(ab')_2$ antibody fragment attached to n more than about 2 polymer molecules, wherein every polymer molecule in the conjugate is at or about 20,000 D to at or about 100,000 D in molecular weight, or is at or about 30,000 D to at or about 100,000 D in molecular weight, or is at or about 40,000 D to at or about 100,000 D in molecular weight, and wherein every polymer molecule in the conjugate is attached to a cysteine residue in the light or heavy chain of the antibody fragment that would ordinarily form the disulfide bridge linking the light and heavy chains, wherein the disulfide bridge is avoided by substituting another amino acid, such as serine, for the corresponding cysteine residue in the opposite chain.

10 In another embodiment, the conjugate contains a $F(ab')_2$ antibody fragment attached to no more than about 2 polymer molecules, wherein every polymer molecule in the conjugate is at or about 20,000 D to at or about 70,000 D in molecular weight, or is at or about 30,000 D to at or about 70,000 D in molecular weight, or is at or about 40,000 D to at or about 70,000 D in molecular weight, and wherein every polymer molecule in the conjugate is attached to a cysteine residue in the light or heavy chain of the antibody
15 fragment that would ordinarily form the disulfide bridge linking the light and heavy chains, wherein the disulfide bridge is avoided by substituting another amino acid, such as serine, for the corresponding cysteine residue in the opposite chain.

In another embodiment, the conjugate contains a $F(ab')_2$ antibody fragment attached to no more than about 2 polymer molecules, wherein every polymer molecule in the conjugate is at or about 20,000 D to at or about 50,000 D in molecular weight, or is at or about 30,000 D to at or about 50,000 D in molecular weight, or is at or about 40,000 D to at or about 50,000 D in molecular weight, and wherein every polymer molecule in the conjugate is attached to a cysteine residue in the light or heavy chain of the antibody fragment that would ordinarily form the disulfide bridge linking the light and heavy chains, wherein the disulfide bridge is avoided by substituting another amino acid, such as serine, for the corresponding cysteine
25 residue in the opposite chain.

In another embodiment, the conjugate contains a $F(ab')_2$ antibody fragment attached to no more than about 2 polymer molecules, wherein every polymer molecule in the conjugate is at or about 20,000 D to at or about 40,000 D in molecular weight, or is at or about 30,000 D to at or about 40,000 D in molecular weight, and wherein every polymer molecule in the conjugate is attached to a cysteine residue in the light or heavy chain of the antibody fragment that would ordinarily form the disulfide bridge linking the light and heavy chains, wherein the disulfide bridge is avoided by substituting another amino acid, such as serine, for the corresponding cysteine residue in the opposite chain.

In yet another embodiment, the conjugate contains an antibody fragment selected from the group consisting of Fab, Fab', and Fab'-SH, wherein the antibody fragment is attached to no more than 1 polymer molecule, wherein the polymer molecule is at least about 20,000 D in molecular weight, or at least about 30,000 D in molecular weight, or at least about 40,000 D in molecular weight, wherein the polymer
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molecule is attached to a cysteine residue in the light or heavy chain of the antibody fragment that would ordinarily form the disulfide bridge linking the light and heavy chains, wherein the disulfide bridge is avoided by substituting another amino acid, such as serine, for the corresponding cysteine residue in the opposite chain.

- 5 In another embodiment, the conjugate contains an antibody fragment selected from the group consisting of Fab, Fab', and Fab'-SH, wherein the antibody fragment is attached to no more than 1 polymer molecule, wherein the polymer molecule is at or about 20,000 D to at or about 300,000 D in molecular weight, or is at or about 30,000 D to at or about 300,000 D in molecular weight, or is at or about 40,000 D to at or about 300,000 D in molecular weight, wherein the polymer molecule is attached to a cysteine residue in the light or heavy chain of the antibody fragment that would ordinarily form the disulfide bridge linking the light and heavy chains, wherein the disulfide bridge is avoided by substituting another amino acid, such as serine, for the corresponding cysteine residue in the opposite chain.

- 10 In another embodiment, the conjugate contains an antibody fragment selected from the group consisting of Fab, Fab', and Fab'-SH, wherein the antibody fragment is attached to no more than 1 polymer molecule, wherein the polymer molecule is at or about 20,000 D to at or about 100,000 D in molecular weight, or is at or about 30,000 D to at or about 100,000 D in molecular weight, or is at or about 40,000 D to at or about 100,000 D in molecular weight, wherein the polymer molecule is attached to a cysteine residue in the light or heavy chain of the antibody fragment that would ordinarily form the disulfide bridge linking the light and heavy chains, wherein the disulfide bridge is avoided by substituting another amino acid, such as serine, for the corresponding cysteine residue in the opposite chain.

- 15 In another embodiment, the conjugate contains an antibody fragment selected from the group consisting of Fab, Fab', and Fab'-SH, wherein the antibody fragment is attached to no more than 1 polymer molecule, wherein the polymer molecule is at or about 20,000 D to at or about 70,000 D in molecular weight, or is at or about 30,000 D to at or about 70,000 D in molecular weight, or is at or about 40,000 D to at or about 70,000 D in molecular weight, wherein the polymer molecule is attached to a cysteine residue in the light or heavy chain of the antibody fragment that would ordinarily form the disulfide bridge linking the light and heavy chains, wherein the disulfide bridge is avoided by substituting another amino acid, such as serine, for the corresponding cysteine residue in the opposite chain.

- 20 In another embodiment, the conjugate contains an antibody fragment selected from the group consisting of Fab, Fab', and Fab'-SH, wherein the antibody fragment is attached to no more than 1 polymer molecule, wherein the polymer molecule is at or about 20,000 D to at or about 50,000 D in molecular weight, or is at or about 30,000 D to at or about 50,000 D in molecular weight, or is at or about 40,000 D to at or about 50,000 D in molecular weight, wherein the polymer molecule is attached to a cysteine residue in the light or heavy chain of the antibody fragment that would ordinarily form the disulfide bridge linking the light and heavy chains, wherein the disulfide bridge is avoided by substituting another amino acid, such as serine, for the corresponding cysteine residue in the opposite chain.

- 25 In another embodiment, the conjugate contains an antibody fragment selected from the group

consisting of Fab, Fab', and Fab'-SH, wherein the antibody fragment is attached to no more than 1 polymer molecule, wherein the polymer molecule is at or about 20,000 D to at or about 40,000 D in molecular weight, or is at or about 30,000 D to at or about 40,000 D in molecular weight, wherein the polymer molecule is attached to a cysteine residue in the light or heavy chain of the antibody fragment that would ordinarily form the disulfide bridge linking the light and heavy chains, wherein the disulfide bridge is avoided by substituting another amino acid, such as serine, for the corresponding cysteine residue in the opposite chain.

In still another embodiment, the conjugate contains an antibody fragment selected from the group consisting of Fab, Fab', and Fab'-SH, wherein the antibody fragment is attached to no more than 1 polymer molecule, wherein the polymer molecule is at least about 20,000 D in molecular weight, or at least about 30,000 D in molecular weight, or at least about 40,000 D in molecular weight, and wherein the polymer molecule is attached to the hinge region of the antibody fragment.

In another embodiment, the conjugate contains an antibody fragment selected from the group consisting of Fab, Fab', and Fab'-SH, wherein the antibody fragment is attached to no more than 1 polymer molecule, wherein the polymer molecule is at or about 20,000 D to at or about 300,000 D in molecular weight, or is at or about 30,000 D to at or about 300,000 D in molecular weight, or is at or about 40,000 D to at or about 300,000 D in molecular weight, and wherein the polymer molecule is attached to the hinge region of the antibody fragment.

In another embodiment, the conjugate contains an antibody fragment selected from the group consisting of Fab, Fab', and Fab'-SH, wherein the antibody fragment is attached to no more than 1 polymer molecule, wherein the polymer molecule is at or about 20,000 D to at or about 100,000 D in molecular weight, or is at or about 30,000 D to at or about 100,000 D in molecular weight, or is at or about 40,000 D to at or about 100,000 D in molecular weight, and wherein the polymer molecule is attached to the hinge region of the antibody fragment.

In another embodiment, the conjugate contains an antibody fragment selected from the group consisting of Fab, Fab', and Fab'-SH, wherein the antibody fragment is attached to no more than 1 polymer molecule, wherein the polymer molecule is at or about 20,000 D to at or about 70,000 D in molecular weight, or is at or about 30,000 D to at or about 70,000 D in molecular weight, or is at or about 40,000 D to at or about 70,000 D in molecular weight, and wherein the polymer molecule is attached to the hinge region of the antibody fragment.

In another embodiment, the conjugate contains an antibody fragment selected from the group consisting of Fab, Fab', and Fab'-SH, wherein the antibody fragment is attached to no more than 1 polymer molecule, wherein the polymer molecule is at or about 20,000 D to at or about 50,000 D in molecular weight, or is at or about 30,000 D to at or about 50,000 D in molecular weight, or is at or about 40,000 D to at or about 50,000 D in molecular weight, and wherein the polymer molecule is attached to the hinge region of the antibody fragment.

In another embodiment, the conjugate contains an antibody fragment selected from the group

consisting of Fab, Fab', and Fab'-SH, wherein the antibody fragment is attached to no more than 1 polymer molecule, wherein the polymer molecule is at or about 20,000 D to at or about 40,000 D in molecular weight, or is at or about 30,000 D to at or about 40,000 D in molecular weight, and wherein the polymer molecule is attached to the hinge region of the antibody fragment.

5 Although any type of polymer is contemplated for use in constructing the conjugates of the invention, including the polymers and chemical linkage systems described in Section (II)(1)(b) below, polyethylene glycol (PEG) polymers are preferred for use herein.

 In one embodiment, the conjugate is an antibody fragment covalently attached to at least one PEG having an actual MW of at least about 20,000 D.

10 In another embodiment, the conjugate is an antibody fragment covalently attached to at least one PEG having an actual MW of at least about 30,000 D.

 In yet another embodiment, the conjugate is an antibody fragment covalently attached to at least one PEG having an actual MW of at least about 40,000 D.

 In another embodiment, the conjugate is an antibody fragment covalently attached to at least one PEG having an actual MW that is at or about 20,000 D to at or about 300,000 D, or is at or about 30,000 D to at or about 300,000 D, or is at or about 40,000 D to at or about 300,000 D.

 In another embodiment, the conjugate is an antibody fragment covalently attached to at least one PEG having an actual MW that is at or about 20,000 D to at or about 100,000 D, or is at or about 30,000 D to at or about 100,000 D, or is at or about 40,000 D to at or about 100,000 D.

20 ~~In another embodiment, the conjugate is an antibody fragment covalently attached to at least one PEG having an actual MW that is at or about 20,000 D to at or about 70,000 D, or is at or about 30,000 D to at or about 70,000 D, or is at or about 40,000 D to at or about 70,000 D.~~

 In another embodiment, the conjugate is an antibody fragment covalently attached to at least one PEG having an actual MW that is at or about 20,000 D to at or about 50,000 D, or is at or about 30,000 D to at or about 50,000 D, or is at or about 40,000 D to at or about 50,000 D.

25 In another embodiment, the conjugate is an antibody fragment covalently attached to at least one PEG having an actual MW that is at or about 20,000 D to at or about 40,000 D, or is at or about 30,000 D to at or about 40,000 D.

 In another aspect, the invention encompasses a conjugate with a PEG to antibody fragment molar ratio of no more than about 10:1, or no more than about 5:1, or no more than about 4:1, or no more than about 3:1, or no more than about 2:1, or no more than 1:1.

 In yet another aspect, the invention encompasses a conjugate wherein the antibody fragment is attached to about 10 or fewer PEG molecules, each PEG molecule having a molecular weight of at least about 20,000 D, or at least about 30,000 D, or at least about 40,000 D. In another embodiment, the conjugate contains an antibody fragment attached to about 5 or fewer PEG molecules, each PEG molecule having a molecular weight of at least about 20,000 D, or at least about 30,000 D, or at least about 40,000 D.

35 ~~In still another embodiment, the conjugate contains an antibody fragment attached to about 4 or fewer PEG~~

molecules, each PEG molecule having a molecular weight f at least about 20,000 D, or at least about 30,000 D, r at least about 40,000 D. In a further embodiment, the conjugate contains an antibody fragment attached to about 3 or fewer PEG molecules, each PEG molecule having a molecular weight of at least about 20,000 D, or at least about 30,000 D, or at least about 40,000 D. In an additional embodiment, the
5 conjugate contains an antibody fragment attached to about 2 or fewer PEG molecules, each PEG molecule having a molecular weight of at least about 20,000 D, or at least about 30,000 D, or at least about 40,000 D. Also provided herein is a conjugate containing an antibody fragment attached to a single PEG molecule having a molecular weight of at least about 20,000 D, or at least about 30,000 D, or at least about 40,000 D.

In another aspect, the invention encompasses a conjugate wherein the antibody fragment is
10 derivatized with PEG, wherein every PEG molecule in the conjugate is at or about 20,000 D to at or about 300,000 D in molecular weight, or is at or about 30,000 D to at or about 300,000 D in molecular weight, or is at or about 40,000 D to at or about 300,000 D in molecular weight, and wherein the conjugate contains no more than about 10 PEG molecules, or no more than about 5 PEG molecules, or no more than about 4 PEG molecules, or no more than about 3 PEG molecules, or no more than about 2 PEG molecules, or no more
15 than 1 PEG molecule.

In another aspect, the invention encompasses a conjugate wherein the antibody fragment is derivatized with PEG, wherein every PEG molecule in the conjugate is at or about 20,000 D to at or about 100,000 D in molecular weight, or is at or about 30,000 D to at or about 100,000 D in molecular weight, or is at or about 40,000 D to at or about 100,000 D in molecular weight, and wherein the conjugate contains no
20 more than about 10 PEG molecules, or no more than about 5 PEG molecules, or no more than about 4 PEG molecules, or no more than about 3 PEG molecules, or no more than about 2 PEG molecules, or no more than 1 PEG molecule.

In another aspect, the invention encompasses a conjugate wherein the antibody fragment is derivatized with PEG, wherein every PEG molecule in the conjugate is at or about 20,000 D to at or about
25 70,000 D in molecular weight, or is at or about 30,000 D to at or about 70,000 D in molecular weight, or is at or about 40,000 D to at or about 70,000 D in molecular weight, and wherein the conjugate contains no more than about 10 PEG molecules, or no more than about 5 PEG molecules, or no more than about 4 PEG molecules, or no more than about 3 PEG molecules, or no more than about 2 PEG molecules, or no more than 1 PEG molecule.

In another aspect, the invention encompasses a conjugate wherein the antibody fragment is derivatized with PEG, wherein every PEG molecule in the conjugate is at or about 20,000 D to at or about
30 50,000 D in molecular weight, or is at or about 30,000 D to at or about 50,000 D in molecular weight, or is at or about 40,000 D to at or about 50,000 D in molecular weight, and wherein the conjugate contains no more than about 10 PEG molecules, or no more than about 5 PEG molecules, or no more than about 4 PEG molecules, or no more than about 3 PEG molecules, or no more than about 2 PEG molecules, or no more
35 than 1 PEG molecule.

In another aspect, the invention encompasses a conjugate wherein the antibody fragment is

derivatized with PEG, wherein every PEG molecule in the conjugate is at or about 20,000 D to at or about 40,000 D in molecular weight, or is at or about 30,000 D to at or about 40,000 D in molecular weight, and wherein the conjugate contains no more than about 10 PEG molecules, or no more than about 5 PEG molecules, or no more than about 4 PEG molecules, or no more than about 3 PEG molecules, or no more than about 2 PEG molecules, or no more than 1 PEG molecule.

In still another aspect, the invention encompasses a conjugate containing an antibody fragment selected from the group consisting of Fab, Fab', Fab'-SH and F(ab')₂, wherein the antibody fragment is attached to about 10 or fewer PEG molecules, each PEG molecule having a molecular weight of at least about 20,000 D, or at least about 30,000 D, or at least about 40,000 D. In another embodiment, the foregoing conjugate contains an antibody fragment attached to about 5 or fewer PEG molecules, each PEG molecule having a molecular weight of at least about 20,000 D, or at least about 30,000 D, or at least about 40,000 D. In still another embodiment, the foregoing conjugate contains an antibody fragment attached to about 4 or fewer PEG molecules, each PEG molecule having a molecular weight of at least about 20,000 D, or at least about 30,000 D, or at least about 40,000 D. In a further embodiment, the foregoing conjugate contains an antibody fragment attached to about 3 or fewer PEG molecules, each PEG molecule having a molecular weight of at least about 20,000 D, or at least about 30,000 D, or at least about 40,000 D. In an additional embodiment, the foregoing conjugate contains an antibody fragment attached to about 2 or fewer PEG molecules, each PEG molecule having a molecular weight of at least about 20,000 D, or at least about 30,000 D, or at least about 40,000 D. Also provided herein is the foregoing conjugate that contains an antibody fragment attached to a single PEG molecule having a molecular weight of at least about 20,000 D, or at least about 30,000 D, or at least about 40,000 D.

In another aspect, the invention encompasses a conjugate containing an antibody fragment selected from the group consisting of Fab, Fab', Fab'-SH and F(ab')₂, wherein the antibody fragment is derivatized with PEG, wherein every PEG molecule in the conjugate is at or about 20,000 D to at or about 300,000 D in molecular weight, or is at or about 30,000 D to at or about 300,000 D in molecular weight, or is at or about 40,000 D to at or about 300,000 D in molecular weight, and wherein the conjugate contains no more than about 10 PEG molecules, or no more than about 5 PEG molecules, or no more than about 4 PEG molecules, or no more than about 3 PEG molecules, or no more than about 2 PEG molecules, or no more than 1 PEG molecule.

In another aspect, the invention encompasses a conjugate containing an antibody fragment selected from the group consisting of Fab, Fab', Fab'-SH and F(ab')₂, wherein the antibody fragment is derivatized with PEG, wherein every PEG molecule in the conjugate is at or about 20,000 D to at or about 100,000 D in molecular weight, or is at or about 30,000 D to at or about 100,000 D in molecular weight, or is at or about 40,000 D to at or about 100,000 D in molecular weight, and wherein the conjugate contains no more than about 10 PEG molecules, or no more than about 5 PEG molecules, or no more than about 4 PEG molecules, or no more than about 3 PEG molecules, or no more than about 2 PEG molecules, or no more than 1 PEG molecule.

molecule.

In another aspect, the invention encompasses a conjugate containing an antibody fragment selected from the group consisting of Fab, Fab', Fab'-SH and F(ab')₂, wherein the antibody fragment is derivatized with PEG, wherein every PEG molecule in the conjugate is at or about 20,000 D to at or about 70,000 D in molecular weight, or is at or about 30,000 D to at or about 70,000 D in molecular weight, or is at or about 40,000 D to at or about 70,000 D in molecular weight, and wherein the conjugate contains no more than about 10 PEG molecules, or no more than about 5 PEG molecules, or no more than about 4 PEG molecules, or no more than about 3 PEG molecules, or no more than about 2 PEG molecules, or no more than 1 PEG molecule.

In another aspect, the invention encompasses a conjugate containing an antibody fragment selected from the group consisting of Fab, Fab', Fab'-SH and F(ab')₂, wherein the antibody fragment is derivatized with PEG, wherein every PEG molecule in the conjugate is at or about 20,000 D to at or about 50,000 D in molecular weight, or is at or about 30,000 D to at or about 50,000 D in molecular weight, or is at or about 40,000 D to at or about 50,000 D in molecular weight, and wherein the conjugate contains no more than about 10 PEG molecules, or no more than about 5 PEG molecules, or no more than about 4 PEG molecules, or no more than about 3 PEG molecules, or no more than about 2 PEG molecules, or no more than 1 PEG molecule.

In another aspect, the invention encompasses a conjugate containing an antibody fragment selected from the group consisting of Fab, Fab', Fab'-SH and F(ab')₂, wherein the antibody fragment is derivatized with PEG, wherein every PEG molecule in the conjugate is at or about 20,000 D to at or about 40,000 D in molecular weight, or is at or about 30,000 D to at or about 40,000 D in molecular weight, and wherein the conjugate contains no more than about 10 PEG molecules, or no more than about 5 PEG molecules, or no more than about 4 PEG molecules, or no more than about 3 PEG molecules, or no more than about 2 PEG molecules, or no more than 1 PEG molecule.

In a preferred embodiment, the conjugate contains an antibody fragment selected from the group consisting of Fab, Fab', and Fab'-SH, wherein the antibody fragment is derivatized with PEG having a molecular weight of at least about 20,000D, or at least about 30,000D, or at least about 40,000D, and wherein every PEG molecule in the conjugate is attached to the hinge region of the antibody fragment.

In another preferred embodiment, the conjugate contains an antibody fragment selected from the group consisting of Fab, Fab', and Fab'-SH, wherein the antibody fragment is derivatized with PEG having a molecular weight that is at or about 20,000 D to about 300,000 D, or is at or about 30,000 D to at or about 300,000 D, or is at or about 40,000 D to at or about 300,000 D, and wherein every PEG molecule in the conjugate is attached to the hinge region of the antibody fragment.

In another preferred embodiment, the conjugate contains an antibody fragment selected from the group consisting of Fab, Fab', and Fab'-SH, wherein the antibody fragment is derivatized with PEG having a molecular weight that is at or about 20,000 D to about 100,000 D, or is at or about 30,000 D to at or about

100,000 D, or is at r about 40,000 D to at or about 100,000 D, and wherein every PEG molecule in the conjugate is attached to the hinge region of the antibody fragment.

In another preferred embodiment, the conjugate contains an antibody fragment selected from the group consisting of Fab, Fab', and Fab'-SH, wherein the antibody fragment is derivatized with PEG having
5 a molecular weight that is at or about 20,000 D to about 70,000 D, or is at or about 30,000 D to at or about 70,000 D, or is at or about 40,000 D to at or about 70,000 D, and wherein every PEG molecule in the conjugate is attached to the hinge region of the antibody fragment.

In another preferred embodiment, the conjugate contains an antibody fragment selected from the group consisting of Fab, Fab', and Fab'-SH, wherein the antibody fragment is derivatized with PEG having
10 a molecular weight that is at or about 20,000 D to about 50,000 D, or is at or about 30,000 D to at or about 50,000 D, or is at or about 40,000 D to at or about 50,000 D, and wherein every PEG molecule in the conjugate is attached to the hinge region of the antibody fragment.

In another preferred embodiment, the conjugate contains an antibody fragment selected from the group consisting of Fab, Fab', and Fab'-SH, wherein the antibody fragment is derivatized with PEG having
15 a molecular weight that is at or about 20,000 D to about 40,000 D, or is at or about 30,000 D to at or about 40,000 D, and wherein every PEG molecule in the conjugate is attached to the hinge region of the antibody fragment.

In another preferred embodiment, the conjugate contains an antibody fragment selected from the group consisting of Fab, Fab', and Fab'-SH, wherein the antibody fragment is derivatized with PEG,
20 wherein every PEG molecule in the conjugate is at least about 20,000D in molecular weight, or at least about 30,000D in molecular weight, or at least about 40,000D in molecular weight, wherein every PEG molecule in the conjugate molecule is attached to the hinge region of the antibody fragment, and wherein the conjugate contains no more than about 10 PEG molecules, or no more than about 5 PEG molecules, or no more than about 4 PEG molecules, or no more than about 3 PEG molecules, or no more than about 2
25 PEG molecules, or no more than 1 PEG molecule.

In another preferred embodiment, the conjugate contains an antibody fragment selected from the group consisting of Fab, Fab', and Fab'-SH, wherein the antibody fragment is derivatized with PEG, wherein every PEG molecule in the conjugate is at or about 20,000 D to at or about 300,000 D in molecular weight, or is at or about 30,000 D to at or about 300,000 D in molecular weight, or is at or about 40,000 D
30 to at or about 300,000 D in molecular weight, wherein every PEG molecule in the conjugate molecule is attached to the hinge region of the antibody fragment, and wherein the conjugate contains no more than about 10 PEG molecules, or no more than about 5 PEG molecules, or no more than about 4 PEG molecules, or no more than about 3 PEG molecules, or no more than about 2 PEG molecules, or no more than 1 PEG molecule.

In another preferred embodiment, the conjugate contains an antibody fragment selected from the group consisting of Fab, Fab', and Fab'-SH, wherein the antibody fragment is derivatized with PEG,
35 wherein every PEG molecule in the conjugate is at r about 20,000 D to at or about 100,000 D in molecular

weight, or is at or about 30,000 D to at or about 100,000 D in molecular weight, or is at or about 40,000 D to at or about 100,000 D in molecular weight, wherein every PEG molecule in the conjugate molecule is attached to the hinge region of the antibody fragment, and wherein the conjugate contains no more than about 10 PEG molecules, or no more than about 5 PEG molecules, or no more than about 4 PEG molecules, or no more than about 3 PEG molecules, or no more than about 2 PEG molecules, or no more than 1 PEG molecule.

In another preferred embodiment, the conjugate contains an antibody fragment selected from the group consisting of Fab, Fab', and Fab'-SH, wherein the antibody fragment is derivatized with PEG, wherein every PEG molecule in the conjugate is at or about 20,000 D to at or about 70,000 D in molecular weight, or is at or about 30,000 D to at or about 70,000 D in molecular weight, or is at or about 40,000 D to at or about 70,000 D in molecular weight, wherein every PEG molecule in the conjugate molecule is attached to the hinge region of the antibody fragment, and wherein the conjugate contains no more than about 10 PEG molecules, or no more than about 5 PEG molecules, or no more than about 4 PEG molecules, or no more than about 3 PEG molecules, or no more than about 2 PEG molecules, or no more than 1 PEG molecule.

In another preferred embodiment, the conjugate contains an antibody fragment selected from the group consisting of Fab, Fab', and Fab'-SH, wherein the antibody fragment is derivatized with PEG, wherein every PEG molecule in the conjugate is at or about 20,000 D to at or about 50,000 D in molecular weight, or is at or about 30,000 D to at or about 50,000 D in molecular weight, or is at or about 40,000 D to at or about 50,000 D in molecular weight, wherein every PEG molecule in the conjugate molecule is attached to the hinge region of the antibody fragment, and wherein the conjugate contains no more than about 10 PEG molecules, or no more than about 5 PEG molecules, or no more than about 4 PEG molecules, or no more than about 3 PEG molecules, or no more than about 2 PEG molecules, or no more than 1 PEG molecule.

In another preferred embodiment, the conjugate contains an antibody fragment selected from the group consisting of Fab, Fab', and Fab'-SH, wherein the antibody fragment is derivatized with PEG, wherein every PEG molecule in the conjugate is at or about 20,000 D to at or about 40,000 D in molecular weight, or is at or about 30,000 D to at or about 40,000 D in molecular weight, wherein every PEG molecule in the conjugate molecule is attached to the hinge region of the antibody fragment, and wherein the conjugate contains no more than about 10 PEG molecules, or no more than about 5 PEG molecules, or no more than about 4 PEG molecules, or no more than about 3 PEG molecules, or no more than about 2 PEG molecules, or no more than 1 PEG molecule.

In yet another preferred embodiment, the conjugate contains a $F(ab')_2$ antibody fragment derivatized with PEG, wherein every PEG molecule in the conjugate is at least about 20,000D in molecular weight, or at least about 30,000D in molecular weight, or at least about 40,000D in molecular weight, wherein the antibody fragment is attached to no more than about 2 PEG molecules, and wherein every PEG molecule is attached to a cysteine residue in the light or heavy chain of the antibody fragment that would

ordinarily form the disulfide bridge linking the light and heavy chains, wherein the disulfide bridge is avoided by substituting another amino acid, such as serine, for the corresponding cysteine residue in the opposite chain.

5 In another preferred embodiment, the conjugate contains a $F(ab')_2$ antibody fragment derivatized with PEG, wherein every PEG molecule in the conjugate is at or about 20,000 D to at or about 300,000 D in molecular weight, or is at or about 30,000 D to at or about 300,000 D in molecular weight, or is at or about 40,000 D to at or about 300,000 D in molecular weight, wherein the antibody fragment is attached to no more than about 2 PEG molecules, and wherein every PEG molecule is attached to a cysteine residue in the light or heavy chain of the antibody fragment that would ordinarily form the disulfide bridge linking the
10 light and heavy chains, wherein the disulfide bridge is avoided by substituting another amino acid, such as serine, for the corresponding cysteine residue in the opposite chain.

In another preferred embodiment, the conjugate contains a $F(ab')_2$ antibody fragment derivatized with PEG, wherein every PEG molecule in the conjugate is at or about 20,000 D to at or about 100,000 D in molecular weight, or is at or about 30,000 D to at or about 100,000 D in molecular weight, or is at or about
15 40,000 D to at or about 100,000 D in molecular weight, wherein the antibody fragment is attached to no more than about 2 PEG molecules, and wherein every PEG molecule is attached to a cysteine residue in the light or heavy chain of the antibody fragment that would ordinarily form the disulfide bridge linking the light and heavy chains, wherein the disulfide bridge is avoided by substituting another amino acid, such as serine, for the corresponding cysteine residue in the opposite chain.

20 In another preferred embodiment, the conjugate contains a $F(ab')_2$ antibody fragment derivatized with PEG, wherein every PEG molecule in the conjugate is at or about 20,000 D to at or about 70,000 D in molecular weight, or is at or about 30,000 D to at or about 70,000 D in molecular weight, or is at or about 40,000 D to at or about 70,000 D in molecular weight, wherein the antibody fragment is attached to no more than about 2 PEG molecules, and wherein every PEG molecule is attached to a cysteine residue in the light
25 or heavy chain of the antibody fragment that would ordinarily form the disulfide bridge linking the light and heavy chains, wherein the disulfide bridge is avoided by substituting another amino acid, such as serine, for the corresponding cysteine residue in the opposite chain.

In another preferred embodiment, the conjugate contains a $F(ab')_2$ antibody fragment derivatized with PEG, wherein every PEG molecule in the conjugate is at or about 20,000 D to at or about 50,000 D in molecular weight, or is at or about 30,000 D to at or about 50,000 D in molecular weight, or is at or about
30 40,000 D to at or about 50,000 D in molecular weight, wherein the antibody fragment is attached to no more than about 2 PEG molecules, and wherein every PEG molecule is attached to a cysteine residue in the light or heavy chain of the antibody fragment that would ordinarily form the disulfide bridge linking the light and heavy chains, wherein the disulfide bridge is avoided by substituting another amino acid, such as serine, for
35 the corresponding cysteine residue in the opposite chain.

In another preferred embodiment, the conjugate contains a $F(ab')_2$ antibody fragment derivatized

with PEG, wherein every PEG molecule in the conjugate is at or about 20,000 D to at or about 40,000 D in molecular weight, or is at or about 30,000 D to at or about 40,000 D in molecular weight, wherein the antibody fragment is attached to no more than about 2 PEG molecules, and wherein every PEG molecule is attached to a cysteine residue in the light or heavy chain of the antibody fragment that would ordinarily form the disulfide bridge linking the light and heavy chains, wherein the disulfide bridge is avoided by substituting another amino acid, such as serine, for the corresponding cysteine residue in the opposite chain.

In still another preferred embodiment, the conjugate contains an antibody fragment selected from the group consisting of Fab, Fab', and Fab'-SH, wherein the antibody fragment is derivatized with PEG, wherein every PEG molecule in the conjugate is at least about 20,000 D in molecular weight, or at least about 30,000 in molecular weight, or at least about 40,000 D in molecular weight, wherein the antibody fragment is attached to no more than 1 PEG molecule, and wherein the PEG molecule is attached to a cysteine residue in the light or heavy chain of the antibody fragment that would ordinarily form the disulfide bridge linking the light and heavy chains, wherein the disulfide bridge is avoided by substituting another amino acid, such as serine, for the corresponding cysteine residue in the opposite chain.

In another preferred embodiment, the conjugate contains an antibody fragment selected from the group consisting of Fab, Fab', and Fab'-SH, wherein the antibody fragment is derivatized with PEG, wherein every PEG molecule in the conjugate is at or about 20,000 D to at or about 300,000 D in molecular weight, or is at or about 30,000 D to at or about 300,000 D in molecular weight, or is at or about 40,000 D to at or about 300,000 D in molecular weight, wherein the antibody fragment is attached to no more than 1 PEG molecule, and wherein the PEG molecule is attached to a cysteine residue in the light or heavy chain of the antibody fragment that would ordinarily form the disulfide bridge linking the light and heavy chains, wherein the disulfide bridge is avoided by substituting another amino acid, such as serine, for the corresponding cysteine residue in the opposite chain.

In another preferred embodiment, the conjugate contains an antibody fragment selected from the group consisting of Fab, Fab', and Fab'-SH, wherein the antibody fragment is derivatized with PEG, wherein every PEG molecule in the conjugate is at or about 20,000 D to at or about 100,000 D in molecular weight, or is at or about 30,000 D to at or about 100,000 D in molecular weight, or is at or about 40,000 D to at or about 100,000 D in molecular weight, wherein the antibody fragment is attached to no more than 1 PEG molecule, and wherein the PEG molecule is attached to a cysteine residue in the light or heavy chain of the antibody fragment that would ordinarily form the disulfide bridge linking the light and heavy chains, wherein the disulfide bridge is avoided by substituting another amino acid, such as serine, for the corresponding cysteine residue in the opposite chain.

In another preferred embodiment, the conjugate contains an antibody fragment selected from the group consisting of Fab, Fab', and Fab'-SH, wherein the antibody fragment is derivatized with PEG, wherein every PEG molecule in the conjugate is at or about 20,000 D to at or about 70,000 D in molecular weight, or is at or about 30,000 D to at or about 70,000 D in molecular weight, or is at or about 40,000 D to at or about 70,000 D in molecular weight, wherein the antibody fragment is attached to no more than 1 PEG

molecule, and wherein the PEG molecule is attached to a cysteine residue in the light or heavy chain of the antibody fragment that would ordinarily form the disulfide bridge linking the light and heavy chains, wherein the disulfide bridge is avoided by substituting another amino acid, such as serine, for the corresponding cysteine residue in the opposite chain.

5 In another preferred embodiment, the conjugate contains an antibody fragment selected from the group consisting of Fab, Fab', and Fab'-SH, wherein the antibody fragment is derivatized with PEG, wherein every PEG molecule in the conjugate is at or about 20,000 D to at or about 50,000 D in molecular weight, or is at or about 30,000 D to at or about 50,000 D in molecular weight, or is at or about 40,000 D to at or about 50,000 D in molecular weight, wherein the antibody fragment is attached to no more than 1 PEG
10 molecule, and wherein the PEG molecule is attached to a cysteine residue in the light or heavy chain of the antibody fragment that would ordinarily form the disulfide bridge linking the light and heavy chains, wherein the disulfide bridge is avoided by substituting another amino acid, such as serine, for the corresponding cysteine residue in the opposite chain.

In another preferred embodiment, the conjugate contains an antibody fragment selected from the
15 group consisting of Fab, Fab', and Fab'-SH, wherein the antibody fragment is derivatized with PEG, wherein every PEG molecule in the conjugate is at or about 20,000 D to at or about 40,000 D in molecular weight, or is at or about 30,000 D to at or about 40,000 D in molecular weight, wherein the antibody fragment is attached to no more than 1 PEG molecule, and wherein the PEG molecule is attached to a cysteine residue in the light or heavy chain of the antibody fragment that would ordinarily form the disulfide
20 bridge linking the light and heavy chains, wherein the disulfide bridge is avoided by substituting another amino acid, such as serine, for the corresponding cysteine residue in the opposite chain.

It will be appreciated that all of the above-described embodiments of the invention utilizing PEG polymers include conjugates wherein the PEG polymer(s) is (are) linear or branched. In a preferred
embodiment, the conjugate contains an antibody fragment selected from the group consisting of Fab, Fab',
25 and Fab'-SH, wherein the antibody fragment is attached to no more than 1 PEG molecule, and wherein the PEG molecule is branched and at least about 40,000 D in molecular weight. In a particularly surprising and unexpected finding, the inventors discovered that the foregoing conjugate exhibits a serum half-life, MRT and serum clearance rate approaching that of full length antibody as shown in Example X below.

In another preferred embodiment, the conjugate contains an antibody fragment selected from the
30 group consisting of Fab, Fab', and Fab'-SH, wherein the antibody fragment is attached to no more than 1 PEG molecule, and wherein the PEG molecule is branched and has a molecular weight that is at or about 40,000 D to at or about 300,000 D.

In another preferred embodiment, the conjugate contains an antibody fragment selected from the
group consisting of Fab, Fab', and Fab'-SH, wherein the antibody fragment is attached to no more than 1
35 PEG molecule, and wherein the PEG molecule is branched and has a molecular weight that is at or about 40,000 D to at or about 100,000 D.

In another preferred embodiment, the conjugate contains an antibody fragment selected from the

group consisting of Fab, Fab', and Fab'-SH, wherein the antibody fragment is attached to no more than 1 PEG molecule, and wherein the PEG molecule is branched and has a molecular weight that is at or about 40,000 D to at or about 70,000 D.

5 In another preferred embodiment, the conjugate contains an antibody fragment selected from the group consisting of Fab, Fab', and Fab'-SH, wherein the antibody fragment is attached to no more than 1 PEG molecule, and wherein the PEG molecule is branched and has a molecular weight that is at or about 40,000 D to at or about 50,000 D.

10 In another preferred embodiment, the invention provides a conjugate containing an antibody fragment selected from the group consisting of Fab, Fab', and Fab'-SH, wherein the antibody fragment is attached to no more than 1 PEG molecule, wherein the PEG molecule is branched and at least 40,000D in molecular weight, and the PEG molecule is attached to the hinge region of the antibody fragment.

15 In another preferred embodiment, the invention provides a conjugate containing an antibody fragment selected from the group consisting of Fab, Fab', and Fab'-SH, wherein the antibody fragment is attached to no more than 1 PEG molecule, wherein the PEG molecule is branched and has a molecular weight that is at or about 40,000 D to at or about 300,000 D, and the PEG molecule is attached to the hinge region of the antibody fragment.

20 In another preferred embodiment, the invention provides a conjugate containing an antibody fragment selected from the group consisting of Fab, Fab', and Fab'-SH, wherein the antibody fragment is attached to no more than 1 PEG molecule, wherein the PEG molecule is branched and has a molecular weight that is at or about 40,000 D to at or about 100,000 D, and the PEG molecule is attached to the hinge region of the antibody fragment.

25 In another preferred embodiment, the invention provides a conjugate containing an antibody fragment selected from the group consisting of Fab, Fab', and Fab'-SH, wherein the antibody fragment is attached to no more than 1 PEG molecule, wherein the PEG molecule is branched and has a molecular weight that is at or about 40,000 D to at or about 70,000 D, and the PEG molecule is attached to the hinge region of the antibody fragment.

30 In another preferred embodiment, the invention provides a conjugate containing an antibody fragment selected from the group consisting of Fab, Fab', and Fab'-SH, wherein the antibody fragment is attached to no more than 1 PEG molecule, wherein the PEG molecule is branched and has a molecular weight that is at or about 40,000 D to at or about 50,000 D, and the PEG molecule is attached to the hinge region of the antibody fragment.

35 In one aspect, the invention provides any of the above-described conjugates wherein the conjugate contains no more than one antibody fragment. Additionally provided herein is any of the above-described conjugates wherein the conjugate contains one or more antibody fragment(s) covalently linked to one or more polymer molecule(s), such as conjugates containing two or more antibody fragments covalently linked together by polymer molecule(s). In one embodiment, a polymer molecule is used to link together two antibody fragments to form a dumbbell-shaped structure. Also encompassed herein are conjugates formed

by more than two antibody fragments joined by polymer molecule(s) to form a rosette or other shapes. The antibody fragments in such structures can be of the same or different fragment type and can have the same antigen specificity or have different antigen specificities. Such structures can be made by using a polymer molecule derivatized with multiple functional groups permitting the direct attachment, or the attachment by means of bi- or multi-functional linkers, of two or more antibody fragments to the polymer backbone.

In another aspect, the invention encompasses any of the above-described conjugates utilizing an antibody fragment comprising an antigen recognition site that binds to rabbit IL-8 and/or human IL-8. In yet another aspect, the invention encompasses any of the above-described conjugates utilizing an antibody fragment comprising 6G4.2.5LV/L1N35A or 6G4.2.5LV/L1N35E as defined below. In still another aspect, the invention encompasses any of the above-described conjugates utilizing an antibody fragment comprising 6G4.5.2.5HV11 as defined below. In a further aspect, the invention encompasses any of the above-described conjugates utilizing an antibody fragment comprising hu6G4.2.5LV/L1N35A or hu6G4.2.5LV/L1N35E as defined below. In an additional aspect, the invention encompasses any of the above-described conjugates utilizing an antibody fragment comprising hu6G4.2.5HV. Further encompassed herein are any of the above-described conjugates utilizing an antibody fragment comprising 6G4.2.5LV/L1N35A or 6G4.2.5LV/L1N35E and further comprising the CDRs of 6G4.2.5HV as defined below. Also encompassed herein are any of the above described conjugates utilizing an antibody fragment comprising hu6G4.2.5LV/L1N35A or hu6G4.2.5LV/L1N35E and further comprising hu6G4.2.5HV as defined below. Additionally encompassed herein are any of the above-described conjugates utilizing an antibody fragment comprising 6G4.2.5LV11N35A or 6G4.2.5LV11N35E as defined below. Further provided herein are any of the above-described conjugates utilizing an antibody fragment comprising 6G4.2.5LV11N35A or 6G4.2.5LV11N35E and further comprising 6G4.2.5HV11 as defined below.

a. Production of Antibody Fragments

Antibody fragments can be produced by any method known in the art. Generally, an antibody fragment is derived from a parental intact antibody. The parental antibody can be generated by raising polyclonal sera against the desired antigen by multiple subcutaneous (sc) or intraperitoneal (ip) injections of antigen and an adjuvant, such as monophosphoryl lipid A (MPL)/trehalose dicrynomycolate (TDM) (Ribi Immunochem. Research, Inc., Hamilton, MT), at multiple sites. Two weeks later the animals are boosted. 7 to 14 days later animals are bled and the serum is assayed for anti-antigen titer. Animals are boosted until titer plateaus. Sera are harvested from animals, and polyclonal antibodies are isolated from sera by conventional immunoglobulin purification procedures, such as protein A-Sepharose chromatography, hydroxylapatite chromatography, gel filtration, dialysis, or antigen affinity chromatography. The desired antibody fragments can be generated from purified polyclonal antibody preparations by conventional enzymatic methods, e.g. F(ab')₂ fragments are produced by pepsin cleavage of intact antibody, and Fab fragments are produced by briefly digesting intact antibody with papain.

Alternatively, antibody fragments are derived from monoclonal antibodies generated against the desired antigen. Monoclonal antibodies may be made using the hybridoma method first described by Kohler

et al., *Nature*, 256:495 (1975), or may be made by recombinant DNA methods (U.S. Patent No. 4,816,567).

In the hybridoma method, a mouse or other appropriate host animal, such as a hamster or macaque monkey, is immunized as hereinabove described to elicit lymphocytes that produce or are capable of producing antibodies that will specifically bind to the protein used for immunization. Alternatively, 5 lymphocytes may be immunized *in vitro*. Lymphocytes then are fused with myeloma cells using a suitable fusing agent, such as polyethylene glycol, to form a hybridoma cell (Goding, *Monoclonal Antibodies: Principles and Practice*, pp.59-103 (Academic Press, 1986)).

The hybridoma cells thus prepared are seeded and grown in a suitable culture medium that preferably contains one or more substances that inhibit the growth or survival of the unfused, parental 10 myeloma cells. For example, if the parental myeloma cells lack the enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRT or HPRT), the culture medium for the hybridomas typically will include hypoxanthine, aminopterin, and thymidine (HAT medium), which substances prevent the growth of HGPRT-deficient cells.

Preferred myeloma cells are those that fuse efficiently, support stable high-level production of 15 antibody by the selected antibody-producing cells, and are sensitive to a medium such as HAT medium. Among these, preferred myeloma cell lines are murine myeloma lines, such as those derived from MOP-21 and M.C.-11 mouse tumors available from the Salk Institute Cell Distribution Center, San Diego, California USA, and SP-2 or X63-Ag8-653 cells available from the American Type Culture Collection, Rockville, Maryland USA. Human myeloma and mouse-human heteromyeloma cell lines also have been described for 20 the production of human monoclonal antibodies (Kozbor, *J. Immunol.*, 133:3001 (1984); Brodeur *et al.*, *Monoclonal Antibody Production Techniques and Applications*, pp. 51-63 (Marcel Dekker, Inc., New York, 1987)).

Culture medium in which hybridoma cells are growing is assayed for production of monoclonal antibodies directed against the antigen. Preferably, the binding specificity of monoclonal antibodies 25 produced by hybridoma cells is determined by immunoprecipitation or by an *in vitro* binding assay, such as radioimmunoassay (RIA) or enzyme-linked immunoabsorbent assay (ELISA).

The binding affinity of the monoclonal antibody can, for example, be determined by the Scatchard analysis of Munson *et al.*, *Anal. Biochem.*, 107:220 (1980).

After hybridoma cells are identified that produce antibodies of the desired specificity, affinity, 30 and/or activity, the clones may be subcloned by limiting dilution procedures and grown by standard methods (Goding, *Monoclonal Antibodies: Principles and Practice*, pp.59-103 (Academic Press, 1986)). Suitable culture media for this purpose include, for example, D-MEM or RPMI-1640 medium. In addition, the hybridoma cells may be grown *in vivo* as ascites tumors in an animal.

The monoclonal antibodies secreted by the subclones are suitably separated from the culture 35 medium, ascites fluid, or serum by conventional immunoglobulin purification procedures such as, for example, protein A-Sepharose, hydroxylapatite chromatography, gel electrophoresis, dialysis, or affinity chromatography.

DNA encoding the monoclonal antibodies is readily isolated and sequenced using conventional procedures (e.g., by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of the monoclonal antibodies). The hybridoma cells serve as a preferred source of such DNA. Once isolated, the DNA may be placed into expression vectors, which are then transfected into host cells such as *E. coli* cells, simian COS cells, Chinese hamster ovary (CHO) cells, or myeloma cells that do not otherwise produce immunoglobulin protein, to obtain the synthesis of monoclonal antibodies in the recombinant host cells. Review articles on recombinant expression in bacteria of antibody-encoding DNA include Skerra et al., Curr. Opinion in Immunol., 5: 256 (1993) and Pluckthun, Immunol. Revs., 130: 151 (1992).

In a preferred embodiment, the antibody fragment is derived from a humanized antibody. Methods for humanizing non-human antibodies are well known in the art. Generally, a humanized antibody has one or more amino acid residues introduced into it from a source which is non-human. These non-human amino acid residues are often referred to as "import" residues, which are typically taken from an "import" variable domain. It will be appreciated that variable domain sequences obtained from any non-human animal phage display library-derived Fv clone or from any non-human animal hybridoma-derived antibody clone provided as described herein can serve as the "import" variable domain used in the construction of the humanized antibodies of the invention. Humanization can be essentially performed following the method of Winter and co-workers (Jones et al., *Nature*, 321: 522 (1986); Riechmann et al., *Nature*, 332: 323 (1988); Verhoeven et al., *Science*, 239: 1534 (1988)), by substituting non-human animal, e.g. rodent, CDRs or CDR sequences for the corresponding sequences of a human antibody. Accordingly, such "humanized" antibodies are chimeric antibodies (Cabilly et al., *supra*), wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanized antibodies are typically human antibodies in which some CDR residues and possibly some FR residues are substituted by residues from analogous sites in non-human animal, e.g. rodent, antibodies.

The choice of human variable domains, both light and heavy, to be used in making the humanized antibodies is very important to reduce antigenicity. According to the so-called "best-fit" method, the sequence of the variable domain of a non-human animal, e.g. rodent, antibody is screened against the entire library of known human variable-domain sequences. The human sequence which is closest to that of the non-human animal is then accepted as the human framework (FR) for the humanized antibody (Sims et al., *J. Immunol.*, 151: 2296 (1993); Chothia and Lesk, *J. Mol. Biol.*, 196: 901 (1987)). Another method uses a particular framework derived from the consensus sequence of all human antibodies of a particular subgroup light or heavy chains. The same framework can be used for several different humanized antibodies (Carter et al., *Proc. Natl. Acad. Sci USA*, 89: 4285 (1992); Presta et al., *J. Immunol.*, 151: 2623 (1993)).

It is also important that antibodies be humanized with retention of high affinity for the antigen and other favorable biological properties. To achieve this goal, according to a preferred method, humanized antibodies are prepared by a process of analysis of the parental sequences and various conceptual humanized products using three-dimensional models of the parental and humanized sequences. Three-dimensional

immunoglobulin models are commonly available and are familiar to those skilled in the art. Computer programs are available which illustrate and display probable three-dimensional conformational structures of selected candidate immunoglobulin sequences. Inspection of these displays permits analysis of the likely role of the residues in the functioning of the candidate immunoglobulin sequence, i.e., the analysis of residues that influence the ability of the candidate immunoglobulin to bind to its antigen. In this way, FR residues can be selected and combined from the consensus and import sequences so that the desired antibody characteristic, such as increased affinity for the target antigen(s), is achieved. In general, the CDR residues are directly and most substantially involved in influencing antigen binding.

In addition, antibody fragments for use herein can be derived from human monoclonal antibodies. Human monoclonal antibodies against the antigen of interest can be made by the hybridoma method. Human myeloma and mouse-human heteromyeloma cell lines for the production of human monoclonal antibodies have been described, for example, by Kozbor *J. Immunol.*, 133: 3001 (1984); Brodeur *et al.*, *Monoclonal Antibody Production Techniques and Applications*, pp. 51-63 (Marcel Dekker, Inc., New York, 1987); and Boerner *et al.*, *J. Immunol.*, 147: 86 (1991).

It is now possible to produce transgenic animals (e.g. mice) that are capable, upon immunization, of producing a full repertoire of human antibodies in the absence of endogenous immunoglobulin production. For example, it has been described that the homozygous deletion of the antibody heavy-chain joining region (JH) gene in chimeric and germ-line mutant mice results in complete inhibition of endogenous antibody production. Transfer of the human germ-line immunoglobulin gene array in such germ-line mutant mice will result in the production of human antibodies upon antigen challenge. See, e.g., Jakobovits *et al.*, *Proc. Natl. Acad. Sci USA*, 90: 2551 (1993); Jakobovits *et al.*, *Nature*, 362: 255 (1993); Bruggemann *et al.*, *Year in Immunol.*, 7: 33 (1993).

Alternatively, phage display technology (McCafferty *et al.*, *Nature* 348:552 (1990)) can be used to produce human antibodies and antibody fragments in vitro, from immunoglobulin variable (V) domain gene repertoires from unimmunized donors. According to this technique, antibody V domain genes are cloned in-frame into either a major or minor coat protein gene of a filamentous bacteriophage, such as M13 or fd, and displayed as functional antibody fragments on the surface of the phage particle. Because the filamentous particle contains a single-stranded DNA copy of the phage genome, selections based on the functional properties of the antibody also result in selection of the gene encoding the antibody exhibiting those properties. Thus, the phage mimics some of the properties of the B-cell. Phage display can be performed in a variety of formats; for their review see, e.g., Johnson *et al.*, *Current Opinion in Structural Biology* 3:564 (1993). Several sources of V-gene segments can be used for phage display. Clackson *et al.*, *Nature* 352:624 (1991) isolated a diverse array of anti-oxazolone antibodies from a small random combinatorial library of V genes derived from the spleens of immunized mice. A repertoire of V genes from unimmunized human donors can be constructed and antibodies to a diverse array of antigens (including self-antigens) can be isolated essentially following the techniques described by Marks *et al.*, *J. Mol. Biol.* 222:581 (1991), or Griffith *et al.*, *EMBO J.* 12:725 (1993).

In a natural immune response, antibody genes accumulate mutations at a high rate (somatic hypermutation). Some of the changes introduced will confer higher affinity, and B cells displaying high-affinity surface immunoglobulin are preferentially replicated and differentiated during subsequent antigen challenge. This natural process can be mimicked by employing the technique known as "chain shuffling" (Marks et al., Bio/Technol. 10:779 (1992)). In this method, the affinity of "primary" human antibodies obtained by phage display can be improved by sequentially replacing the heavy and light chain V region genes with repertoires of naturally occurring variants (repertoires) of V domain genes obtained from unimmunized donors. This technique allows the production of antibodies and antibody fragments with affinities in the nM range. A strategy for making very large phage antibody repertoires has been described by Waterhouse et al., Nucl. Acids Res. 21:2265 (1993).

Gene shuffling can also be used to derive human antibodies from non-human, e.g. rodent, antibodies, where the human antibody has similar affinities and specificities to the starting non-human antibody. According to this method, which is also called "epitope imprinting", either the heavy or light chain variable region of a non-human antibody fragment obtained by phage display techniques as described above is replaced with a repertoire of human V domain genes, creating a population of non-human chain/human chain scFv or Fab chimeras. Selection with antigen results in isolation of a non-human chain/human chain chimeric scFv or Fab wherein the human chain restores the antigen binding site destroyed upon removal of the corresponding non-human chain in the primary phage display clone, i.e. the epitope governs (imprints) the choice of the human chain partner. When the process is repeated in order to replace the remaining non-human chain, a human antibody is obtained (see PCT WO 93/06213 published April 1, 1993). Unlike traditional humanization of non-human antibodies by CDR grafting, this technique provides completely human antibodies, which have no FR or CDR residues of non-human origin.

The invention also encompasses the use of bispecific and heteroconjugate antibody fragments having specificities for at least two different antigens. Bispecific and heteroconjugate antibodies can be prepared as full length antibodies or as antibody fragments (e.g. F(ab')₂ bispecific antibody fragments). Antibody fragments having more than two valencies (e.g. trivalent or higher valency antibody fragments) are also contemplated for use herein. Bispecific antibodies, heteroconjugate antibodies, and multi-valent antibodies can be prepared as described in Section (II)(3)(C) below.

As described above, DNA encoding the monoclonal antibody or antibody fragment of interest can be isolated from its hybridoma or phage display clone of origin, and then manipulated to create humanized and/or affinity matured constructs. In addition, known techniques can be employed to introduce an amino acid residue or residues into any desired location on the polypeptide backbone of the antibody fragment, e.g. a cysteine residue placed in the hinge region of the heavy chain, thereby providing a site for specific attachment of polymer molecule(s). In one embodiment, the native cysteine residue in either the light or heavy chain of the antibody fragment that would ordinarily form the disulfide bridge linking the light and heavy chains is substituted with another amino acid, such as serine, in order to leave the partner cysteine residue in the opposite chain with a free sulfhydryl for specific attachment of polymer molecule.

Upon construction of the desired antibody or antibody fragment-encoding clone, the clone can be used for recombinant production of the antibody fragment as described in Section (II)(4) below. Finally, the antibody or antibody fragment product can be recovered from host cell culture and purified as described in Section (II)(4)(F) below. In the case of embodiments utilizing an antibody fragment engineered to lack a cysteine residue that ordinarily forms the disulfide bridge between the light and heavy chains as described above, preferred recombinant production systems include bacterial expression and product recovery procedures utilizing the low pH osmotic shock method described in the "Alternative Fab'-SH Purification" section of Example T below. If a full length antibody is produced, the desired antibody fragment can be obtained therefrom by subjecting the intact antibody to enzymatic digestion according to known methods, e.g. as described in Section (II)(4)(G) below.

b. Construction of Antibody Fragment-Polymer Conjugates

The antibody fragment-polymer conjugates of the invention can be made by derivatizing the desired antibody fragment with an inert polymer. It will be appreciated that any inert polymer which provides the conjugate with the desired apparent size or which has the selected actual MW as taught herein is suitable for use in constructing the antibody fragment-polymer conjugates of the invention.

Many inert polymers are suitable for use in pharmaceuticals. See, e.g., Davis et al., Biomedical Polymers: Polymeric Materials and Pharmaceuticals for Biomedical Use, pp.441-451 (1980). In all embodiments of the invention, a non-proteinaceous polymer is used. The nonproteinaceous polymer ordinarily is a hydrophilic synthetic polymer, i.e., a polymer not otherwise found in nature. However, polymers which exist in nature and are produced by recombinant or *in vitro* methods are also useful, as are polymers which are isolated from native sources. Hydrophilic polyvinyl polymers fall within the scope of this invention, e.g. polyvinylalcohol and polyvinylpyrrolidone. Particularly useful are polyalkylene ethers such as polyethylene glycol (PEG); polyoxyalkylenes such as polyoxyethylene, polyoxypropylene, and block copolymers of polyoxyethylene and polyoxypropylene (Pluronics); polymethacrylates; carbomers; branched or unbranched polysaccharides which comprise the saccharide monomers D-mannose, D- and L-galactose, fucose, fructose, D-xylose, L-arabinose, D-glucuronic acid, sialic acid, D-galacturonic acid, D-mannuronic acid (e.g. polymannuronic acid, or alginic acid), D-glucosamine, D-galactosamine, D-glucose and neuraminic acid including homopolysaccharides and heteropolysaccharides such as lactose, amylopectin, starch, hydroxyethyl starch, amylose, dextrane sulfate, dextran, dextrans, glycogen, or the polysaccharide subunit of acid mucopolysaccharides, e.g. hyaluronic acid; polymers of sugar alcohols such as poly sorbitol and polymannitol; heparin or heparan. The polymer prior to cross-linking need not be, but preferably is, water soluble, but the final conjugate must be water soluble. Preferably, the conjugate exhibits a water solubility of at least about 0.01 mg/ml, and more preferably at least about 0.1 mg/ml, and still more preferably at least about 1 mg/ml. In addition, the polymer should not be highly immunogenic in the conjugate form, nor should it possess viscosity that is incompatible with intravenous infusion or injection if the conjugate is intended to be administered by such routes.

In one embodiment, the polymer contains only a single group which is reactive. This helps to

avoid cross-linking of protein molecules. However, it is within the scope herein to maximize reaction conditions to reduce cross-linking, or to purify the reaction products through gel filtration or ion exchange chromatography to recover substantially homogeneous derivatives. In other embodiments, the polymer contains two or more reactive groups for the purpose of linking multiple antibody fragments to the polymer backbone. Again, gel filtration or ion exchange chromatography can be used to recover the desired derivative in substantially homogeneous form.

The molecular weight of the polymer can range up to about 500,000 D, and preferably is at least about 20,000 D, or at least about 30,000 D, or at least about 40,000 D. The molecular weight chosen can depend upon the effective size of the conjugate to be achieved, the nature (e.g. structure, such as linear or branched) of the polymer, and the degree of derivatization, i.e. the number of polymer molecules per antibody fragment, and the polymer attachment site or sites on the antibody fragment.

The polymer can be covalently linked to the antibody fragment through a multifunctional crosslinking agent which reacts with the polymer and one or more amino acid residues of the antibody fragment to be linked. However, it is also within the scope of the invention to directly crosslink the polymer by reacting a derivatized polymer with the antibody fragment, or vice versa.

The covalent crosslinking site on the antibody fragment includes the N-terminal amino group and epsilon amino groups found on lysine residues, as well as other amino, imino, carboxyl, sulfhydryl, hydroxyl or other hydrophilic groups. The polymer may be covalently bonded directly to the antibody fragment without the use of a multifunctional (ordinarily bifunctional) crosslinking agent. Covalent binding to amino groups is accomplished by known chemistries based upon cyanuric chloride, carbonyl diimidazole, aldehyde reactive groups (PEG alkoxide plus diethyl acetal of bromoacetaldehyde; PEG plus DMSO and acetic anhydride, or PEG chloride plus the phenoxide of 4-hydroxybenzaldehyde, activated succinimidyl esters, activated dithiocarbonate PEG, 2,4,5-trichlorophenylchloroformate or P-nitrophenylchloroformate activated PEG.) Carboxyl groups are derivatized by coupling PEG-amine using carbodiimide. Sulfhydryl groups are derivatized by coupling to maleimido-substituted PEG (e.g. alkoxy-PEG amine plus sulfosuccinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate) as described in WO 97/10847 published March 27, 1997, or PEG-maleimide commercially available from Shearwater Polymers, Inc., Huntsville, AL). Alternatively, free amino groups on the antibody fragment (e.g. epsilon amino groups on lysine residues) can be thiolated with 2-imino-thiolane (Traut's reagent) and then coupled to maleimide-containing derivatives of PEG as described in Pedley et al., Br. J. Cancer, 70: 1126-1130 (1994).

The polymer will bear a group which is directly reactive with an amino acid side chain, or the N- or C-terminus of the polypeptide linked, or which is reactive with the multifunctional cross-linking agent. In general, polymers bearing such reactive groups are known for the preparation of immobilized proteins. In order to use such chemistries here, one should employ a water soluble polymer otherwise derivatized in the same fashion as insoluble polymers heretofore employed for protein immobilization. Cyanogen bromide activation is a particularly useful procedure to employ in crosslinking polysaccharides.

"Water soluble" in reference to the starting polymer means that the polymer or its reactive

intermediate used for conjugation is sufficiently water soluble to participate in a derivatization reaction.

The degree of substitution with such a polymer will vary depending upon the number of reactive sites on the antibody fragment, the molecular weight, hydrophilicity and other characteristics of the polymer, and the particular antibody fragment derivatization sites chosen. In general, the conjugate contains from 1 to about 10 polymer molecules, but greater numbers of polymer molecules attached to the antibody fragments of the invention are also contemplated. The desired amount of derivatization is easily achieved by using an experimental matrix in which the time, temperature and other reaction conditions are varied to change the degree of substitution, after which the level of polymer substitution of the conjugates is determined by size exclusion chromatography or other means known in the art.

The polymer, e.g. PEG, is cross-linked to the antibody fragment by a wide variety of methods known *per se* for the covalent modification of proteins with nonproteinaceous polymers such as PEG. Certain of these methods, however, are not preferred for the purposes herein. Cyanuronic chloride chemistry leads to many side reactions, including protein cross-linking. In addition, it may be particularly likely to lead to inactivation of proteins containing sulfhydryl groups. Carbonyl diimidazole chemistry (Beauchamp *et al.*, Anal Biochem. 131, 25-33 [1983]) requires high pH (>8.5), which can inactivate proteins. Moreover, since the "activated PEG" intermediate can react with water, a very large molar excess of "activated PEG" over protein is required. The high concentrations of PEG required for the carbonyl diimidazole chemistry also led to problems in purification, as both gel filtration chromatography and hydrophilic interaction chromatography are adversely affected. In addition, the high concentrations of "activated PEG" may precipitate protein, a problem that *per se* has been noted previously (Davis, U.S. Patent No. 4,179,337). On the other hand, aldehyde chemistry (Royer, U.S. Patent No. 4,002,531) is more efficient since it requires only a 40-fold molar excess of PEG and a 1-2 hr incubation. However, the manganese dioxide suggested by Royer for preparation of the PEG aldehyde is problematic "because of the pronounced tendency of PEG to form complexes with metal-based oxidizing agents" (Harris *et al.*, J. Polym. Sci. Polym. Chem. Ed. 22, 341-52 [1984]). The use of a Moffatt oxidation, utilizing DMSO and acetic anhydride, obviates this problem. In addition, the sodium borohydride suggested by Royer must be used at high pH and has a significant tendency to reduce disulfide bonds. In contrast, sodium cyanoborohydride, which is effective at neutral pH and has very little tendency to reduce disulfide bonds is preferred. In another preferred embodiment, maleimido-activated PEG is used for coupling to free thiols on the antibody fragment.

Functionalized PEG polymers to modify the antibody fragments of the invention are available from Shearwater Polymers, Inc. (Huntsville, AL). Such commercially available PEG derivatives include, but are not limited to, amino-PEG, PEG amino acid esters, PEG-hydrazide, PEG-thiol, PEG-succinate, carboxymethylated PEG, PEG-propionic acid, PEG amino acids, PEG succinimidyl succinate, PEG succinimidyl propionate, succinimidyl ester of carboxymethylated PEG, succinimidyl carbonate of PEG, succinimidyl esters of amino acid-PEGs, PEG-oxycarbonylimidazole, PEG-nitrophenyl carbonate, PEG tresylate, PEG-glycidyl ether, PEG-aldehyde, PEG vinylsulfone, PEG-maleimide, PEG-orthopyridyl-

disulfide, heterofunctional PEGs, PEG vinyl derivatives, PEG silanes, and PEG phospholides. The reaction conditions for coupling these PEG derivatives will vary depending on the protein, the desired degree of PEGylation, and the PEG derivative utilized. Some factors involved in the choice of PEG derivatives include: the desired point of attachment (such as lysine or cysteine R-groups), hydrolytic stability and reactivity of the derivatives, stability, toxicity and antigenicity of the linkage, suitability for analysis, etc. Specific instructions for the use of any particular derivative are available from the manufacturer.

The conjugates of this invention are separated from the unreacted starting materials by gel filtration or ion exchange HPLC. Heterologous species of the conjugates are purified from one another in the same fashion.

The conjugates may also be purified by ion-exchange chromatography. The chemistry of many of the electrophilically activated PEG's results in a reduction of amino group charge of the PEGylated product. Thus, high resolution ion exchange chromatography can be used to separate the free and conjugated proteins, and to resolve species with different levels of PEGylation. In fact, the resolution of different species (e.g. containing one or two PEG residues) is also possible due to the difference in the ionic properties of the unreacted amino acids. In one embodiment, species with difference levels of PEGylation are resolved according to the methods described in WO 96/34015 (International Application No. PCT/US96/05550 published October 31, 1996).

In a preferred embodiment, the conjugate is generated by utilizing the derivatization and purification methods described in Section (T) of the Examples below.

In one aspect, the invention provides any of the above-described conjugates formed by its component parts, i.e. one or more antibody fragment(s) covalently attached to one or more polymer molecule(s), without any extraneous matter in the covalent molecular structure of the conjugate.

c. Other Derivatives of Large Effective Size Conjugates

In another aspect, any of the above-described conjugates can be modified to contain one or more component(s) in addition to the antibody fragment component(s) and polymer component(s) that form the conjugate, wherein the modification does not alter the essential functional property of the conjugate, namely, the substantially improved serum half-life, MRT and/or serum clearance rate as compared to that of the parental antibody fragment from which the conjugate is derived. In one embodiment, the invention provides any of the above-described conjugates modified to incorporate one or more nonproteinaceous functional group(s). For example, the conjugate can be modified to incorporate nonproteinaceous labels or reporter molecules, such as radiolabels, including any radioactive substance used in medical treatment or imaging or used as an effector function or tracer in an animal model, such as radioisotopic labels ^{99}Tc , ^{90}Y , ^{111}In , ^{32}P , ^{14}C , ^{125}I , ^3H , ^{131}I , ^{11}C , ^{15}O , ^{13}N , ^{18}F , ^{35}S , ^{51}Cr , ^{57}Co , ^{226}Ra , ^{60}Co , ^{59}Fe , ^{75}Se , ^{152}Eu , ^{67}Cu , ^{217}Bi , ^{211}At , ^{212}Pb , ^{47}Sc , ^{109}Pd , ^{234}Th , ^{40}K , and the like, non-radioisotopic labels such as ^{157}Gd , ^{55}Mn , ^{52}Fe , etc., fluorescent or chemiluminescent labels, including fluorophores such as rare earth chelates, fluorescein and its derivatives, rhodamine and its derivatives, isothiocyanate, phycoerythrin, phycocyanin,

allophycocyanin, o-phthalaldehyde, fluorescamine, ¹⁵²Eu, dansyl, umbelliferone, luciferin, luminal label, isoluminal label, an aromatic acridinium ester label, an imidazole label, an acridinium salt label, an oxalate ester label, an aequorin label, 2,3-dihydrophthalazinediones, biotin/avidin, spin labels, stable free radicals, and the like.

5 Conventional methods are available to bind these labels covalently to the polypeptide antibody fragment or polymer component of the conjugate. In one aspect, any conjugate of the invention is modified by derivatizing the antibody fragment component with any of the above-described non-proteinaceous labels, wherein the label is directly or indirectly (through a coupling agent) attached to the antibody fragment, and wherein such derivatization of the antibody fragment does not contribute or introduce any polymer moiety
10 into the molecular structure of the conjugate. For instance, coupling agents such as dialdehydes, carbodiimides, dimaleimides, bis-imidates, bis-diazotized benzidine, and the like can be used to tag the antibody fragment with the above-described fluorescent or chemiluminescent labels. See, for example, U.S. Pat. No. 3,940,475 (fluorimetry), Morrison, Meth. Enzymol., 32b, 103 (1974), Svyanen et al., J. Biol. Chem., 284, 3762 (1973), and Bolton and Hunter, Biochem. J., 133, 529 (1973).

15 In the case of embodiments utilizing radiolabels, both direct and indirect labeling can be used to incorporate the selected radionuclide into the conjugate. As used herein in the context of radiolabeling, the phrases "indirect labeling" and "indirect labeling approach" both mean that a chelating agent is covalently attached to the antibody fragment moiety or polymer moiety of the conjugate and at least one radionuclide is inserted into the chelating agent. Preferred chelating agents and radionuclides are set forth in Srivagava,
20 S.C. and Mease, R.C., "Progress in Research on Ligands, Nuclides and Techniques for Labeling Monoclonal Antibodies," Nucl. Med. Bio., 18(6): 589-603 (1991). A particularly preferred chelating agent is 1-isothiocyanatobenzyl-3-methyldiothelene triaminepent acetic acid ("MX-DTPA"). As used herein in the context of radiolabeling, the phrases "direct labeling" and "direct labeling approach" both mean that a radionuclide is covalently attached directly to the antibody fragment moiety (typically via an amino acid
25 residue) or to the polymer moiety of the conjugate. Preferred radionuclides for use in direct labeling of conjugate are provided in Srivagava and Mease, supra. In one embodiment, the conjugate is directly labeled with ¹³¹I covalently attached to tyrosine residues. In another embodiment, the antibody fragment component of the conjugate is directly or indirectly labeled with any of the above-described radiolabels, wherein such labeling of the antibody fragment does not contribute or introduce any polymer moiety into
30 the molecular structure of the conjugate.

d. Therapeutic Compositions and Administration of Large Effective Size Conjugates

 The conjugate of the invention is useful for treating the disease indications that are treated with the parent intact antibody. For example, a conjugate derived from an anti-IL-8 antibody or fragment is useful in the treatment of inflammatory disorders as described in Section (II)(5)(B) below. Therapeutic formulations
35 of the conjugate of the invention can be prepared by utilizing the same procedures described for the formulation of the anti-IL-8 antibodies and fragments of the invention in Section (II)(5)(B) below. The conjugate of the invention can be administered in place of the parent antibody for a given disease indication

by modifying the formulation, dosage, administration protocol, and other aspects of a therapeutic regimen as required by the different pharmacodynamic characteristics of the conjugate and as dictated by common medical knowledge and practice.

e. Reagent Uses for Large Effective Size Conjugates

5 The conjugate of the invention also finds application as a reagent in an animal model system for in vivo study of the biological functions of the antigen recognized by the conjugate. The conjugate would enable the practitioner to inactivate or detect the cognate antigen in circulation or in tissue for a far greater period of time than would be possible with art-known constructs while removing any Fc interaction (which could attend the use of an intact antibody) from the system. In addition, the increased half-life of the
10 conjugate of the invention can be applied advantageously to the induction of tolerance for the underivatized antibody fragment in a test animal by employing the Wie et al., Int. Archs. Allergy Appl. Immunol., 64: 84-99 (1981) method for allergen tolerization, which would permit the practitioner to repeatedly challenge the tolerized animal with the underivatized parental antibody fragment without generating an immune response against the parental fragment.

15 2. HUMANIZED 6G4.2.5 MONOCLONAL ANTIBODIES AND ANTIBODY FRAGMENTS

In one embodiment, the invention provides an antibody fragment or full length antibody comprising a heavy chain comprising the amino acid sequence of amino acids 1-230 (herein referred to as "6G4.2.5HV11") of the humanized anti-IL-8 6G4.2.5v11 heavy chain polypeptide amino acid sequence of Figs. 37A-37B (SEQ ID NO: 75).

20 The invention encompasses a single chain antibody fragment comprising the 6G4.2.5HV11, with or without any additional amino acid sequence. In one embodiment, the invention provides a single chain antibody fragment comprising the 6G4.2.5HV11 without any associated light chain amino acid sequence, i.e. a single chain species that makes up one half of a Fab fragment.

Further provided herein are an antibody or antibody fragment comprising the 6G4.2.5HV11, and
25 further comprising a light chain comprising the amino acid sequence of amino acids 1-219 (herein referred to as "6G4.2.5LV11") of the humanized anti-IL-8 6G4.2.5v11 light chain polypeptide amino acid sequence of Fig. 31B (SEQ ID NO: 65).

In one embodiment, the invention provides a single chain antibody fragment wherein the 6G4.2.5HV11 and the 6G4.2.5LV11 are contained in a single chain polypeptide species. In a preferred
30 embodiment, the single chain antibody fragment comprises the 6G4.2.5HV11 joined to the 6G4.2.5LV11 by means of a flexible peptide linker sequence, wherein the heavy chain and light chain domains can associate in a "dimeric" structure analogous to that formed in a two-chain Fab species. In another embodiment, the single chain antibody fragment is a species comprising the 6G4.2.5HV11 joined to the 6G4.2.5LV11 by a linker that is too short to permit intramolecular pairing of complementary domains, i.e. a single chain
35 polypeptide monomer that forms a diabody upon dimerization with another monomer.

In yet another embodiment, the invention provides an antibody fragment comprising a plurality of polypeptide chains, wherein one polypeptide chain comprises the 6G4.2.5HV11 and a second polypeptide

chain comprises the 6G4.2.SLV11 and the two polypeptide chains are covalently linked by one or more interchain disulfide bonds. In a preferred embodiment, the foregoing two-chain antibody fragment is selected from the group consisting of Fab, Fab', Fab'-SH, and F(ab')₂.

5 The invention also provides an antibody or antibody fragment comprising a heavy chain containing the 6G4.2.5HV11 and optionally further comprising a light chain containing the 6G4.2.SLV11, wherein the heavy chain, and optionally the light chain, is (are) fused to an additional moiety, such as additional immunoglobulin constant domain sequence. Constant domain sequence can be added to the heavy chain and/or light chain sequence(s) to form species with full or partial length heavy and/or light chain(s). It will be appreciated that constant regions of any isotype can be used for this purpose, including IgG, IgM, IgA, 10 IgD, and IgE constant regions, and that such constant regions can be obtained from any human or animal species. Preferably, the constant domain sequence is human in origin. Suitable human constant domain sequences can be obtained from Kabat *et al.* (supra).

In a preferred embodiment, the antibody or antibody fragment comprises the 6G4.2.5HV11 in a heavy chain that is fused to or contains a leucine zipper sequence. The leucine zipper can increase the 15 affinity and/or production efficiency of the antibody or antibody fragment of interest. Suitable leucine zipper sequences include the jun and fos leucine zippers taught by Kostelney *et al.*, *J. Immunol.*, 148: 1547-1553 (1992) and the GCN4 leucine zipper described in the Examples below. In a preferred embodiment, the antibody or antibody fragment comprises the 6G4.2.5HV11 fused at its C-terminus to the GCN4 leucine zipper to yield the amino acid sequence of amino acids 1-275 (herein referred to as "6G4.2.5HV11GCN4") 20 of the heavy chain polypeptide amino acid sequence of Figs. 37A-37B (SEQ ID NO: 75).

3. VARIANTS OF HUMANIZED 6G4.2.5 MONOCLONAL ANTIBODIES AND ANTIBODY FRAGMENTS

The invention additionally encompasses humanized anti-IL-8 monoclonal antibody and antibody fragments comprising variants of the 6G4.2.5 complementarity determining regions (CDRs) or variants of 25 the 6G4.2.5v11 variable domains which exhibit higher affinity for human IL-8 and/or possess properties that yield greater efficiency in recombinant production processes.

A. 6G4.2.5LV VARIANTS

In one aspect, the invention provides humanized anti-IL-8 monoclonal antibodies and antibody fragments comprising the complementarity determining regions (referred to herein as the "CDRs of 30 6G4.2.5LV") L1, L2, and L3 of the 6G4.2.5 light chain variable domain amino acid sequence of Fig. 24, wherein L1 corresponds to amino acids 24-39 of the amino acid sequence of Fig. 24, L2 corresponds to amino acids 55-61 of the amino acid sequence of Fig. 24 (SEQ ID NO: 48), and L3 corresponds to amino acids 94-102 of the amino acid sequence of Fig. 24 (SEQ ID NO: 48).

In addition, the invention provides a variant 6G4.2.5 humanized antibody or antibody fragment 35 comprising a humanized light chain variable domain comprising a variant (hereinafter referred to as "6G4.2.5LV CDRs variant") of the complementarity determining regions L1, L2, and L3 of the 6G4.2.5 variable light chain domain amino acid sequence of Fig. 24 (SEQ ID NO: 48). In one embodiment, the

invention provides a variant 6G4.2.5 humanized antibody or antibody fragment comprising a 6G4.2.5LV CDRs variant (herein referred to as "6G4.2.5LV/L1N35X₃₅") wherein L1 corresponds to amino acids 24-39 of the amino acid sequence of Fig. 24 (SEQ ID NO: 48) with the proviso that any amino acid other than Asn (denoted as "X₃₅") is substituted for Asn at amino acid position 35, L2 corresponds to amino acids 55-61 of the amino acid sequence of Fig. 24 (SEQ ID NO: 48), and L3 corresponds to amino acids 94-102 of the amino acid sequence of Fig. 24 (SEQ ID NO: 48). In a preferred embodiment, the invention provides a variant 6G4.2.5 humanized antibody or antibody fragment comprising a 6G4.2.5LV CDRs variant (herein referred to as "6G4.2.5LV/L1N35A") wherein L1 corresponds to amino acids 24-39 of the amino acid sequence of Fig. 24 (SEQ ID NO: 48) with the proviso that Ala is substituted for Asn at amino acid position 35, L2 corresponds to amino acids 55-61 of the amino acid sequence of Fig. 24 (SEQ ID NO: 48), and L3 corresponds to amino acids 94-102 of the amino acid sequence of Fig. 24 (SEQ ID NO: 48). In another preferred embodiment, the invention provides a variant 6G4.2.5 humanized antibody or antibody fragment comprising a 6G4.2.5LV CDRs variant (herein referred to as "6G4.2.5LV/L1N35E") wherein L1 corresponds to amino acids 24-39 of the amino acid sequence of Fig. 24 (SEQ ID NO: 48) with the proviso that Glu is substituted for Asn at amino acid position 35, L2 corresponds to amino acids 55-61 of the amino acid sequence of Fig. 24 (SEQ ID NO: 48), and L3 corresponds to amino acids 94-102 of the amino acid sequence of Fig. 24 (SEQ ID NO: 48).

In a second aspect, the invention provides a variant 6G4.2.5 humanized antibody or antibody fragment comprising a 6G4.2.5LV CDRs variant (herein referred to as "6G4.2.5LV/L1S26X₂₆") wherein L1 corresponds to amino acids 24-39 of the amino acid sequence of Fig. 24 (SEQ ID NO: 48) with the proviso that any amino acid other than Ser (denoted as "X₂₆") is substituted for Ser at amino acid position 26, L2 corresponds to amino acids 55-61 of the amino acid sequence of Fig. 24 (SEQ ID NO: 48), and L3 corresponds to amino acids 94-102 of the amino acid sequence of Fig. 24 (SEQ ID NO: 48). In a preferred embodiment, the invention provides a variant 6G4.2.5 humanized antibody or antibody fragment comprising a 6G4.2.5LV CDRs variant (herein referred to as "6G4.2.5LV/L1S26A") wherein L1 corresponds to amino acids 24-39 of the amino acid sequence of Fig. 24 (SEQ ID NO: 48) with the proviso that Ala is substituted for Ser at amino acid position 26, L2 corresponds to amino acids 55-61 of the amino acid sequence of Fig. 24 (SEQ ID NO: 48), and L3 corresponds to amino acids 94-102 of the amino acid sequence of Fig. 24 (SEQ ID NO: 48).

In a third aspect, the invention provides a variant 6G4.2.5 humanized antibody or antibody fragment comprising a 6G4.2.5LV CDRs variant (herein referred to as "6G4.2.5LV/L3H98X₉₈") wherein L1 corresponds to amino acids 24-39 of the amino acid sequence of Fig. 24 (SEQ ID NO: 48), L2 corresponds to amino acids 55-61 of the amino acid sequence of Fig. 24 (SEQ ID NO: 48), and L3 corresponds to amino acids 94-102 of the amino acid sequence of Fig. 24 (SEQ ID NO: 48) with the proviso that any amino acid other than His (denoted as "X₉₈") is substituted for His at amino acid position 98. In a

preferred embodiment, the invention provides a variant 6G4.2.5 humanized antibody or antibody fragment comprising a 6G4.2.5LV CDRs variant (herein referred to as "6G4.2.5LV/L3H98A") wherein L1 corresponds to amino acids 24-39 of the amino acid sequence of Fig. 24 (SEQ ID NO: 48), L2 corresponds to amino acids 55-61 of the amino acid sequence of Fig. 24 (SEQ ID NO: 48), and L3 corresponds to amino acids 94-102 of the amino acid sequence of Fig. 24 (SEQ ID NO: 48) with the proviso that Ala is substituted for His at amino acid position 98.

In a fourth aspect, the invention provides a variant 6G4.2.5 humanized antibody or antibody fragment comprising a 6G4.2.5LV CDRs variant (herein referred to as "6G4.2.5LV/L1S26X₂₆,N35X₃₅") wherein L1 corresponds to amino acids 24-39 of the amino acid sequence of Fig. 24 (SEQ ID NO: 48) with the proviso that any amino acid other than Ser (denoted as "X₂₆") is substituted for Ser at amino acid position 26 and any amino acid other than Asn (denoted as "X₃₅") is substituted for Asn at amino acid position 35, L2 corresponds to amino acids 55-61 of the amino acid sequence of Fig. 24 (SEQ ID NO: 48), and L3 corresponds to amino acids 94-102 of the amino acid sequence of Fig. 24 (SEQ ID NO: 48). In a preferred embodiment, the invention provides a variant 6G4.2.5 humanized antibody or antibody fragment comprising a 6G4.2.5LV CDRs variant (herein referred to as "6G4.2.5LV/L1S26A,N35A") wherein L1 corresponds to amino acids 24-39 of the amino acid sequence of Fig. 24 (SEQ ID NO: 48) with the proviso that Ala is substituted for Ser at amino acid position 26 and Ala is substituted for Asn at amino acid position 35, L2 corresponds to amino acids 55-61 of the amino acid sequence of Fig. 24 (SEQ ID NO: 48), and L3 corresponds to amino acids 94-102 of the amino acid sequence of Fig. 24 (SEQ ID NO: 48).

In a fifth aspect, the invention provides a variant 6G4.2.5 humanized antibody or antibody fragment comprising a 6G4.2.5LV CDRs variant (herein referred to as "6G4.2.5LV/L1N35X₃₅/L3H98X₉₈") wherein L1 corresponds to amino acids 24-39 of the amino acid sequence of Fig. 24 (SEQ ID NO: 48) with the proviso that any amino acid other than Asn (denoted as "X₃₅") is substituted for Asn at amino acid position 35, L2 corresponds to amino acids 55-61 of the amino acid sequence of Fig. 24 (SEQ ID NO: 48), and L3 corresponds to amino acids 94-102 of the amino acid sequence of Fig. 24 (SEQ ID NO: 48) with the proviso that any amino acid other than His (denoted as "X₉₈") is substituted for His at amino acid position 98. In a preferred embodiment, the invention provides a variant 6G4.2.5 humanized antibody or antibody fragment comprising a 6G4.2.5LV CDRs variant (herein referred to as "6G4.2.5LV/L1N35A/L3H98A") wherein L1 corresponds to amino acids 24-39 of the amino acid sequence of Fig. 24 (SEQ ID NO: 48) with the proviso that Ala is substituted for Asn at amino acid position 35, L2 corresponds to amino acids 55-61 of the amino acid sequence of Fig. 24 (SEQ ID NO: 48), and L3 corresponds to amino acids 94-102 of the amino acid sequence of Fig. 24 (SEQ ID NO: 48) with the proviso that Ala is substituted for His at amino acid position 98.

In a sixth aspect, the invention provides a variant 6G4.2.5 humanized antibody or antibody fragment comprising a 6G4.2.5LV CDRs variant (herein referred to as "6G4.2.5LV/L1S26X₂₆/L3H98X₉₈")

wherein L1 corresponds to amino acids 24-39 of the amino acid sequence of Fig. 24 (SEQ ID NO: 48) with the proviso that any amino acid other than Ser (denoted as "X₂₆") is substituted for Ser at amino acid position 26, L2 corresponds to amino acids 55-61 of the amino acid sequence of Fig. 24 (SEQ ID NO: 48), and L3 corresponds to amino acids 94-102 of the amino acid sequence of Fig. 24 (SEQ ID NO: 48) with the proviso that any amino acid other than His (denoted as "X₉₈") is substituted for His at amino acid position 98. In a preferred embodiment, the invention provides a variant 6G4.2.5 humanized antibody or antibody fragment comprising a 6G4.2.5LV CDRs variant (herein referred to as "6G4.2.5LV/L1S26A/L3H98A") wherein L1 corresponds to amino acids 24-39 of the amino acid sequence of Fig. 24 (SEQ ID NO: 48) with the proviso that Ala is substituted for Ser at amino acid position 26, L2 corresponds to amino acids 55-61 of the amino acid sequence of Fig. 24 (SEQ ID NO: 48), and L3 corresponds to amino acids 94-102 of the amino acid sequence of Fig. 24 (SEQ ID NO: 48) with the proviso that Ala is substituted for His at amino acid position 98.

In a seventh aspect, the invention provides a variant 6G4.2.5 humanized antibody or antibody fragment comprising a 6G4.2.5LV CDRs variant (here referred to as "6G4.2.5LV/L1S26X₂₆,N35X₃₅/L3H98X₉₈") wherein L1 corresponds to amino acids 24-39 of the amino acid sequence of Fig. 24 (SEQ ID NO: 48) with the proviso that any amino acid other than Ser (denoted as "X₂₆") is substituted for Ser at amino acid position 26 and any amino acid other than Asn (denoted as "X₃₅") is substituted for Asn at amino acid position 35, L2 corresponds to amino acids 55-61 of the amino acid sequence of Fig. 24 (SEQ ID NO: 48), and L3 corresponds to amino acids 94-102 of the amino acid sequence of Fig. 24 (SEQ ID NO: 48) with the proviso that any amino acid other than His (denoted as "X₉₈") is substituted for His at amino acid position 98. In a preferred embodiment, the invention provides a variant 6G4.2.5 humanized antibody or antibody fragment comprising a 6G4.2.5LV CDRs variant (here referred to as "6G4.2.5LV/L1S26A,N35A/L3H98A") wherein L1 corresponds to amino acids 24-39 of the amino acid sequence of Fig. 24 (SEQ ID NO: 48) with the proviso that Ala is substituted for Ser at amino acid position 26 and Ala is substituted for Asn at amino acid position 35, L2 corresponds to amino acids 55-61 of the amino acid sequence of Fig. 24 (SEQ ID NO: 48), and L3 corresponds to amino acids 94-102 of the amino acid sequence of Fig. 24 (SEQ ID NO: 48) with the proviso that Ala is substituted for His at amino acid position 98.

The humanized light chain variable domains of the invention can be constructed by using any of the techniques for antibody humanization known in the art. Humanization can be essentially performed following the method of Winter and co-workers (Jones *et al.*, Nature 321:522 (1986); Riechmann *et al.*, Nature 332:323 (1988); Verhoeven *et al.*, Science 239:1534 (1988)), by substituting the CDRs of 6G4.2.5LV or the CDRs of a 6G4.2.5LV CDRs variant for the corresponding sequences of a human antibody light chain variable domain. Accordingly, such "humanized" derivatives containing the CDRs of 6G4.2.5LV or the CDRs of a 6G4.2.5LV CDRs variant are chimeric (Cabilly *et al.*, *supra*). The humanized

light chain variable domain comprising the CDRs of 6G4.2.5LV or the CDRs of a 6G4.2.5LV CDRs variant can also contain some FR residues that are substituted by residues from analogous sites in the murine 6G4.2.5 antibody light chain variable domain ("6G4.2.5LV"). The complete amino acid sequence of 6G4.2.5LV is set out as amino acids 1-114 of the amino acid sequence of Fig. 24 (SEQ ID NO: 48).

5 The invention further provides a humanized antibody or antibody fragment comprising a humanized light chain variable domain comprising the CDRs of 6G4.2.5LV or the CDRs of a 6G4.2.5LV CDRs variant as described above, and further comprising a humanized heavy chain variable domain comprising the complementarity determining regions (CDRs) H1, H2, and H3 of the 6G4.2.5 (murine monoclonal antibody) variable heavy chain domain amino acid sequence of Fig. 25 (SEQ ID NO: 50),
10 wherein H1 correspond to amino acids 26-35 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50), wherein H2 corresponds to amino acids 50-66 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50), and wherein H3 corresponds to amino acids 99-111 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50). The above-described H1, H2, and H3 CDRs of the 6G4.2.5 heavy chain variable domain ("6G4.2.5HV") are collectively referred to as the "CDRs of 6G4.2.5HV".

15 In another embodiment, the invention provides a humanized antibody or antibody fragment comprising a humanized light chain variable domain comprising the CDRs of 6G4.2.5LV or the CDRs of a 6G4.2.5LV CDRs variant as described above, and further comprising a humanized heavy chain variable domain comprising a variant (herein referred to as a "6G4.2.5HV CDRs variant") of the H1, H2, and H3 CDRs of the 6G4.2.5 (murine monoclonal antibody) variable heavy chain domain amino acid sequence of
20 Fig. 25 (SEQ ID NO: 50). In one 6G4.2.5HV CDRs variant (referred to herein as "6G4.2.5HV/H1S31Z₃₁"), H1 correspond to amino acids 26-35 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that any amino acid other than Ser (denoted as "Z₃₁") is substituted for Ser at amino acid position 31, H2 corresponds to amino acids 50-66 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50), and H3 corresponds to amino acids 99-111 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50). In a preferred
25 6G4.2.5HV CDRs variant (referred to herein as "6G4.2.5HV/H1S31A"), H1 correspond to amino acids 26-35 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Ala is substituted for Ser at amino acid position 31, H2 corresponds to amino acids 50-66 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50), and H3 corresponds to amino acids 99-111 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50).

30 In a second 6G4.2.5HV CDRs variant (referred to herein as "6G4.2.5HV/H2S54Z₅₄"), H1 corresponds to amino acids 26-35 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50), H2 corresponds to amino acids 50-66 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that any amino acid other than Ser (denoted as "Z₅₄") is substituted for Ser at amino acid position 54, and H3 corresponds to amino acids 99-111 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50). In a preferred
35 6G4.2.5HV CDRs variant (referred to herein as "6G4.2.5HV/H2S54A"), H1 corresponds to amino acids 26-35 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50), H2 corresponds to amino acids 50-66 of the

amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Ala is substituted for Ser at amino acid position 54, and H3 corresponds to amino acids 99-111 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50).

5 In a third 6G4.2.5HV CDRs variant (referred to herein as "6G4.2.5HV/H3D100E"), wherein H1 correspond to amino acids 26-35 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50), wherein H2 corresponds to amino acids 50-66 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50), and wherein H3 corresponds to amino acids 99-111 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Glu is substituted for Asp at amino acid position 100.

10 In a fourth 6G4.2.5HV CDRs variant (referred to herein as "6G4.2.5HV/H3R102K"), wherein H1 correspond to amino acids 26-35 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50), wherein H2 corresponds to amino acids 50-66 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50), and wherein H3 corresponds to amino acids 99-111 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Lys is substituted for Arg at amino acid position 102.

15 In a fifth 6G4.2.5HV CDRs variant (referred to herein as "6G4.2.5HV/H3D106E"), wherein H1 correspond to amino acids 26-35 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50), wherein H2 corresponds to amino acids 50-66 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50), and wherein H3 corresponds to amino acids 99-111 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Glu is substituted for Asp at amino acid position 106.

20 In a seventh 6G4.2.5HV CDRs variant (referred to herein as "6G4.2.5HV/H3D100E,R102K"), wherein H1 correspond to amino acids 26-35 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50), wherein H2 corresponds to amino acids 50-66 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50), and wherein H3 corresponds to amino acids 99-111 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Glu is substituted for Asp at amino acid position 100 and Lys is substituted for Arg at amino acid position 102.

25 In an eighth 6G4.2.5HV CDRs variant (referred to herein as "6G4.2.5HV/H3R102K,D106E"), wherein H1 correspond to amino acids 26-35 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50), wherein H2 corresponds to amino acids 50-66 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50), and wherein H3 corresponds to amino acids 99-111 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Lys is substituted for Arg at amino acid position 102 and Glu is substituted for Asp at amino acid position 106.

30 In a ninth 6G4.2.5HV CDRs variant (referred to herein as "6G4.2.5HV/H3D100E,D106E"), wherein H1 correspond to amino acids 26-35 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50), wherein H2 corresponds to amino acids 50-66 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50), and wherein H3 corresponds to amino acids 99-111 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Glu is substituted for Asp at amino acid position 100 and Glu is substituted for Asp at amino acid position 106.

35 In a tenth 6G4.2.5HV CDRs variant (referred to herein as "6G4.2.5HV/H3D100E,R102K,D106E"),

wherein H1 correspond to amino acids 26-35 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50), wherein H2 corresponds to amino acids 50-66 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50), and wherein H3 corresponds to amino acids 99-111 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Glu is substituted for Asp at amino acid position 100, Lys is substituted for Arg at amino acid position 102, and Glu is substituted for Asp at amino acid position 106.

In an eleventh 6G4.2.5HV CDRs variant (referred to herein as "6G4.2.5HV/H1S31Z₃₁/H2S54Z₅₄"), H1 correspond to amino acids 26-35 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that any amino acid other than Ser (denoted as "Z₃₁") is substituted for Ser at amino acid position 31, H2 corresponds to amino acids 50-66 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that any amino acid other than Ser (denoted as "Z₅₄") is substituted for Ser at amino acid position 54, and H3 corresponds to amino acids 99-111 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50). In a preferred 6G4.2.5HV CDRs variant (referred to herein as "6G4.2.5HV/H1S31A/H2S54A"), H1 correspond to amino acids 26-35 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Ala is substituted for Ser at amino acid position 31, H2 corresponds to amino acids 50-66 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Ala is substituted for Ser at amino acid position 54, and H3 corresponds to amino acids 99-111 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50).

In a twelfth 6G4.2.5HV CDRs variant (referred to herein as "6G4.2.5HV/H1S31Z₃₁/H3D100E"), H1 correspond to amino acids 26-35 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that any amino acid other than Ser (denoted as "Z₃₁") is substituted for Ser at amino acid position 31, H2 corresponds to amino acids 50-66 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50), and H3 corresponds to amino acids 99-111 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Glu is substituted for Asp at amino acid position 100. In a preferred 6G4.2.5HV CDRs variant (referred to herein as "6G4.2.5HV/H1S31A/H3D100E"), H1 correspond to amino acids 26-35 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Ala is substituted for Ser at amino acid position 31, H2 corresponds to amino acids 50-66 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50), and H3 corresponds to amino acids 99-111 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Glu is substituted for Asp at amino acid position 100.

In a thirteenth 6G4.2.5HV CDRs variant (referred to herein as "6G4.2.5HV/H1S31Z₃₁/H3R102K"), H1 correspond to amino acids 26-35 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that any amino acid other than Ser (denoted as "Z₃₁") is substituted for Ser at amino acid position 31, H2 corresponds to amino acids 50-66 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50), and H3 corresponds to amino acids 99-111 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Lys is substituted for Arg at amino acid position 102. In a preferred 6G4.2.5HV CDRs variant (referred to herein as "6G4.2.5HV/H1S31A/H3R102K"), H1

correspond to amino acids 26-35 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Ala is substituted for Ser at amino acid position 31, H2 corresponds to amino acids 50-66 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50), and H3 corresponds to amino acids 99-111 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Lys is substituted for Arg at amino acid position 102.

A fourteenth 6G4.2.5HV CDRs variant (referred to herein as "6G4.2.5HV/H1S31Z₃₁/H3D106E"), H1 correspond to amino acids 26-35 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that any amino acid other than Ser (denoted as "Z₃₁") is substituted for Ser at amino acid position 31, H2 corresponds to amino acids 50-66 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50), and H3 corresponds to amino acids 99-111 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Glu is substituted for Asp at amino acid position 106. In a preferred 6G4.2.5HV CDRs variant (referred to herein as "6G4.2.5HV/H1S31A/H3D106E"), H1 correspond to amino acids 26-35 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Ala is substituted for Ser at amino acid position 31, H2 corresponds to amino acids 50-66 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50), and H3 corresponds to amino acids 99-111 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Glu is substituted for Asp at amino acid position 106.

A fifteenth 6G4.2.5HV CDRs variant (referred to herein as "6G4.2.5HV/H1S31Z₃₁/H3D100E,R102K"), H1 correspond to amino acids 26-35 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that any amino acid other than Ser (denoted as "Z₃₁") is substituted for Ser at amino acid position 31, H2 corresponds to amino acids 50-66 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50), and H3 corresponds to amino acids 99-111 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Glu is substituted for Asp at amino acid position 100 and Lys is substituted for Arg at amino acid position 102. In a preferred 6G4.2.5HV CDRs variant (referred to herein as "6G4.2.5HV/H1S31A/H3D100E,R102K"), H1 correspond to amino acids 26-35 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Ala is substituted for Ser at amino acid position 31, H2 corresponds to amino acids 50-66 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50), and H3 corresponds to amino acids 99-111 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Glu is substituted for Asp at amino acid position 100 and Lys is substituted for Arg at amino acid position 102.

In a sixteenth 6G4.2.5HV CDRs variant (referred to herein as "6G4.2.5HV/H1S31Z₃₁/H3R102K,D106E"), H1 correspond to amino acids 26-35 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that any amino acid other than Ser (denoted as "Z₃₁") is substituted for Ser at amino acid position 31, H2 corresponds to amino acids 50-66 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50), and H3 corresponds to amino acids 99-111 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Lys is substituted for Arg at amino acid position 102.

102 and Glu is substituted for Asp at amino acid position 106. In a preferred 6G4.2.5HV CDRs variant (referred to herein as "6G4.2.5HV/H1S31A/H3R102K,D106E"), H1 correspond to amino acids 26-35 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Ala is substituted for Ser at amino acid position 31, H2 corresponds to amino acids 50-66 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50), and H3 corresponds to amino acids 99-111 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Lys is substituted for Arg at amino acid position 102 and Glu is substituted for Asp at amino acid position 106.

In a seventeenth 6G4.2.5HV CDRs variant (referred to herein as "6G4.2.5HV/H1S31Z₃₁/H3D100E,D106E"), H1 correspond to amino acids 26-35 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that any amino acid other than Ser (denoted as "Z₃₁") is substituted for Ser at amino acid position 31, H2 corresponds to amino acids 50-66 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50), and H3 corresponds to amino acids 99-111 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Glu is substituted for Asp at amino acid position 100 and Glu is substituted for Asp at amino acid position 106. In a preferred 6G4.2.5HV CDRs variant (referred to herein as "6G4.2.5HV/H1S31A/H3D100E,D106E"), H1 correspond to amino acids 26-35 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Ala is substituted for Ser at amino acid position 31, H2 corresponds to amino acids 50-66 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50), and H3 corresponds to amino acids 99-111 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Glu is substituted for Asp at amino acid position 100 and Glu is substituted for Asp at amino acid position 106.

In an eighteenth 6G4.2.5HV CDRs variant (referred to herein as "6G4.2.5HV/H1S31Z₃₁/H3D100E,R102K,D106E"), H1 correspond to amino acids 26-35 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that any amino acid other than Ser (denoted as "Z₃₁") is substituted for Ser at amino acid position 31, H2 corresponds to amino acids 50-66 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50), and H3 corresponds to amino acids 99-111 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Glu is substituted for Asp at amino acid position 100, Lys is substituted for Arg at amino acid position 102 and Glu is substituted for Asp at amino acid position 106. In a preferred 6G4.2.5HV CDRs variant (referred to herein as "6G4.2.5HV/H1S31A/H3D100E,R102K,D106E"), H1 correspond to amino acids 26-35 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Ala is substituted for Ser at amino acid position 31, H2 corresponds to amino acids 50-66 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50), and H3 corresponds to amino acids 99-111 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Glu is substituted for Asp at amino acid position 100, Lys is substituted for Arg at amino acid position 102 and Glu is substituted for Asp at amino acid position 106.

In a nineteenth 6G4.2.5HV CDRs variant (referred to herein as "6G4.2.5HV/H2S54Z₅₄/H3D100E"), H1 corresponds to amino acids 26-35 of the amino acid sequence of

Fig. 25 (SEQ ID NO: 50), H2 corresponds to amino acids 50-66 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that any amino acid other than Ser (denoted as "Z₅₄") is substituted for Ser at amino acid position 54, and H3 corresponds to amino acids 99-111 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Glu is substituted for Asp at amino acid position 100. In a preferred 6G4.2.5HV CDRs variant (referred to herein as "6G4.2.5HV/H2S54A/H3D100E"), H1 corresponds to amino acids 26-35 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50), H2 corresponds to amino acids 50-66 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Ala is substituted for Ser at amino acid position 54, and H3 corresponds to amino acids 99-111 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Glu is substituted for Asp at amino acid position 100.

In a twentieth 6G4.2.5HV CDRs variant (referred to herein as "6G4.2.5HV/H2S54Z₅₄/H3R102K"), H1 corresponds to amino acids 26-35 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50), H2 corresponds to amino acids 50-66 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that any amino acid other than Ser (denoted as "Z₅₄") is substituted for Ser at amino acid position 54, and H3 corresponds to amino acids 99-111 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Lys is substituted for Arg at amino acid position 102. In a preferred 6G4.2.5HV CDRs variant (referred to herein as "6G4.2.5HV/H2S54A/H3R102K"), H1 corresponds to amino acids 26-35 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50), H2 corresponds to amino acids 50-66 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Ala is substituted for Ser at amino acid position 54, and H3 corresponds to amino acids 99-111 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Lys is substituted for Arg at amino acid position 102.

In a twenty-first 6G4.2.5HV CDRs variant (referred to herein as "6G4.2.5HV/H2S54Z₅₄/H3D106E"), H1 corresponds to amino acids 26-35 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50), H2 corresponds to amino acids 50-66 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that any amino acid other than Ser (denoted as "Z₅₄") is substituted for Ser at amino acid position 54, and H3 corresponds to amino acids 99-111 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Glu is substituted for Asp at amino acid position 106. In a preferred 6G4.2.5HV CDRs variant (referred to herein as "6G4.2.5HV/H2S54A/H3D106E"), H1 corresponds to amino acids 26-35 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50), H2 corresponds to amino acids 50-66 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Ala is substituted for Ser at amino acid position 54, and H3 corresponds to amino acids 99-111 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Glu is substituted for Asp at amino acid position 106.

In a twenty-second 6G4.2.5HV CDRs variant (referred to herein as "6G4.2.5HV/H2S54Z₅₄/H3D100E,R102K"), H1 corresponds to amino acids 26-35 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50), H2 corresponds to amino acids 50-66 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that any amino acid other than Ser (denoted as "Z₅₄") is

substituted for Ser at amino acid position 54, and H3 corresponds to amino acids 99-111 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Glu is substituted for Asp at amino acid position 100 and Lys is substituted for Arg at amino acid position 102. In a preferred 6G4.2.5HV CDRs variant (referred to herein as "6G4.2.5HV/H2S54A/H3D100E,R102K"), H1 corresponds to amino acids 26-35 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50), H2 corresponds to amino acids 50-66 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Ala is substituted for Ser at amino acid position 54, and H3 corresponds to amino acids 99-111 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Glu is substituted for Asp at amino acid position 100 and Lys is substituted for Arg at amino acid position 102.

In a twenty-third 6G4.2.5HV CDRs variant (referred to herein as "6G4.2.5HV/H2S54Z₅₄/H3R102K,D106E"), H1 corresponds to amino acids 26-35 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50), H2 corresponds to amino acids 50-66 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that any amino acid other than Ser (denoted as "Z₅₄") is substituted for Ser at amino acid position 54, and H3 corresponds to amino acids 99-111 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Lys is substituted for Arg at amino acid position 102 and Glu is substituted for Asp at amino acid position 106. In a preferred 6G4.2.5HV CDRs variant (referred to herein as "6G4.2.5HV/H2S54A/H3R102K,D106E"), H1 corresponds to amino acids 26-35 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50), H2 corresponds to amino acids 50-66 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Ala is substituted for Ser at amino acid position 54, and H3 corresponds to amino acids 99-111 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Lys is substituted for Arg at amino acid position 102 and Glu is substituted for Asp at amino acid position 106.

In a twenty-fourth 6G4.2.5HV CDRs variant (referred to herein as "6G4.2.5HV/H2S54Z₅₄/H3D100E,D106E"), H1 corresponds to amino acids 26-35 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50), H2 corresponds to amino acids 50-66 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that any amino acid other than Ser (denoted as "Z₅₄") is substituted for Ser at amino acid position 54, and H3 corresponds to amino acids 99-111 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Glu is substituted for Asp at amino acid position 100 and Glu is substituted for Asp at amino acid position 106. In a preferred 6G4.2.5HV CDRs variant (referred to herein as "6G4.2.5HV/H2S54A/H3D100E,D106E"), H1 corresponds to amino acids 26-35 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50), H2 corresponds to amino acids 50-66 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Ala is substituted for Ser at amino acid position 54, and H3 corresponds to amino acids 99-111 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Glu is substituted for Asp at amino acid position 100 and Glu is substituted for Asp at amino acid position 106.

In a twenty-fifth 6G4.2.5HV CDRs variant (referred to herein as

"6G4.2.5HV/H2S54Z₅₄/H3D100E,R102K,D106E"), H1 corresponds to amino acids 26-35 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50), H2 corresponds to amino acids 50-66 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that any amino acid other than Ser (denoted as "Z₅₄") is substituted for Ser at amino acid position 54, and H3 corresponds to amino acids 99-111 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Glu is substituted for Asp at amino acid position 100, Lys is substituted for Arg at amino acid position 102 and Glu is substituted for Asp at amino acid position 106. In a preferred 6G4.2.5HV CDRs variant (referred to herein as "6G4.2.5HV/H2S54A/H3D100E,R102K,D106E"), H1 corresponds to amino acids 26-35 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50), H2 corresponds to amino acids 50-66 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Ala is substituted for Ser at amino acid position 54, and H3 corresponds to amino acids 99-111 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Glu is substituted for Asp at amino acid position 100, Lys is substituted for Arg at amino acid position 102 and Glu is substituted for Asp at amino acid position 106.

In a twenty-sixth 6G4.2.5HV CDRs variant (referred to herein as "6G4.2.5HV/H1S31Z₃₁/H2S54Z₅₄/H3D100E"), H1 correspond to amino acids 26-35 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that any amino acid other than Ser (denoted as "Z₃₁") is substituted for Ser at amino acid position 31, H2 corresponds to amino acids 50-66 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that any amino acid other than Ser (denoted as "Z₅₄") is substituted for Ser at amino acid position 54, and H3 corresponds to amino acids 99-111 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Glu is substituted for Asp at amino acid position 100. In a preferred 6G4.2.5HV CDRs variant (referred to herein as "6G4.2.5HV/H1S31A/H2S54A/H3D100E"), H1 correspond to amino acids 26-35 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Ala is substituted for Ser at amino acid position 31, H2 corresponds to amino acids 50-66 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Ala is substituted for Ser at amino acid position 54, and H3 corresponds to amino acids 99-111 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Glu is substituted for Asp at amino acid position 100.

In a twenty-seventh 6G4.2.5HV CDRs variant (referred to herein as "6G4.2.5HV/H1S31Z₃₁/H2S54Z₅₄/H3R102K"), H1 correspond to amino acids 26-35 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that any amino acid other than Ser (denoted as "Z₃₁") is substituted for Ser at amino acid position 31, H2 corresponds to amino acids 50-66 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that any amino acid other than Ser (denoted as "Z₅₄") is substituted for Ser at amino acid position 54, and H3 corresponds to amino acids 99-111 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Lys is substituted for Arg at amino

acid position 102. In a preferred 6G4.2.5HV CDRs variant (referred to herein as "6G4.2.5HV/H1S31A/H2S54A/H3R102K"), H1 correspond to amino acids 26-35 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Ala is substituted for Ser at amino acid position 31, H2 corresponds to amino acids 50-66 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Ala is substituted for Ser at amino acid position 54, and H3 corresponds to amino acids 99-111 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Lys is substituted for Arg at amino acid position 102.

In a twenty-eighth 6G4.2.5HV CDRs variant (referred to herein as "6G4.2.5HV/H1S31Z₃₁/H2S54Z₅₄/H3D106E"), H1 correspond to amino acids 26-35 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that any amino acid other than Ser (denoted as "Z₃₁") is substituted for Ser at amino acid position 31, H2 corresponds to amino acids 50-66 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that any amino acid other than Ser (denoted as "Z₅₄") is substituted for Ser at amino acid position 54, and H3 corresponds to amino acids 99-111 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Glu is substituted for Asp at amino acid position 106. In a preferred 6G4.2.5HV CDRs variant (referred to herein as "6G4.2.5HV/H1S31A/H2S54A/H3D106E"), H1 correspond to amino acids 26-35 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Ala is substituted for Ser at amino acid position 31, H2 corresponds to amino acids 50-66 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Ala is substituted for Ser at amino acid position 54, and H3 corresponds to amino acids 99-111 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Glu is substituted for Asp at amino acid position 106.

In a twenty-ninth 6G4.2.5HV CDRs variant (referred to herein as "6G4.2.5HV/H1S31Z₃₁/H2S54Z₅₄/H3D100E,R102K"), H1 correspond to amino acids 26-35 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that any amino acid other than Ser (denoted as "Z₃₁") is substituted for Ser at amino acid position 31, H2 corresponds to amino acids 50-66 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that any amino acid other than Ser (denoted as "Z₅₄") is substituted for Ser at amino acid position 54, and H3 corresponds to amino acids 99-111 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Glu is substituted for Asp at amino acid position 100 and Lys is substituted for Arg at amino acid position 102. In a preferred 6G4.2.5HV CDRs variant (referred to herein as "6G4.2.5HV/H1S31A/H2S54A/H3D100E,R102K"), H1 correspond to amino acids 26-35 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Ala is substituted for Ser at amino acid position 31, H2 corresponds to amino acids 50-66 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Ala is substituted for Ser at amino acid position 54, and H3 corresponds to amino acids 99-111 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Glu is substituted for Asp at amino acid position 100 and Lys is substituted for Arg at amino

acid position 102.

In a thirtieth 6G4.2.5HV CDRs variant (referred to herein as "6G4.2.5HV/H1S31Z₃₁/H2S54Z₅₄/H3R102K,D106E"), H1 correspond to amino acids 26-35 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that any amino acid other than Ser (denoted as "Z₃₁") is substituted for Ser at amino acid position 31, H2 corresponds to amino acids 50-66 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that any amino acid other than Ser (denoted as "Z₅₄") is substituted for Ser at amino acid position 54, and H3 corresponds to amino acids 99-111 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Lys is substituted for Arg at amino acid position 102 and Glu is substituted for Asp at amino acid position 106. In a preferred 6G4.2.5HV CDRs variant (referred to herein as "6G4.2.5HV/H1S31A/H2S54A/H3R102K,D106E"), H1 correspond to amino acids 26-35 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Ala is substituted for Ser at amino acid position 31, H2 corresponds to amino acids 50-66 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Ala is substituted for Ser at amino acid position 54, and H3 corresponds to amino acids 99-111 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Lys is substituted for Arg at amino acid position 102 and Glu is substituted for Asp at amino acid position 106.

In a thirty-first 6G4.2.5HV CDRs variant (referred to herein as "6G4.2.5HV/H1S31Z₃₁/H2S54Z₅₄/H3D100E,D106E"), H1 correspond to amino acids 26-35 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that any amino acid other than Ser (denoted as "Z₃₁") is substituted for Ser at amino acid position 31, H2 corresponds to amino acids 50-66 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that any amino acid other than Ser (denoted as "Z₅₄") is substituted for Ser at amino acid position 54, and H3 corresponds to amino acids 99-111 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Glu is substituted for Asp at amino acid position 100 and Glu is substituted for Asp at amino acid position 106. In a preferred 6G4.2.5HV CDRs variant (referred to herein as "6G4.2.5HV/H1S31A/H2S54A/H3D100E,D106E"), H1 correspond to amino acids 26-35 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Ala is substituted for Ser at amino acid position 31, H2 corresponds to amino acids 50-66 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Ala is substituted for Ser at amino acid position 54, and H3 corresponds to amino acids 99-111 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Glu is substituted for Asp at amino acid position 100 and Glu is substituted for Asp at amino acid position 106.

In a thirty-second 6G4.2.5HV CDRs variant (referred to herein as "6G4.2.5HV/H1S31Z₃₁/H2S54Z₅₄/H3D100E,R102K,D106E"), H1 correspond to amino acids 26-35 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that any amino acid other than Ser

(denoted as "Z₃₁") is substituted for Ser at amino acid position 31, H2 corresponds to amino acids 50-66 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that any amino acid other than Ser (denoted as "Z₅₄") is substituted for Ser at amino acid position 54, and H3 corresponds to amino acids 99-111 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Glu is substituted for Asp at amino acid position 100, Lys is substituted for Arg at amino acid position 102 and Glu is substituted for Asp at amino acid position 106. In a preferred 6G4.2.5HV CDRs variant (referred to herein as "6G4.2.5HV/H1S31A/H2S54A/H3D100E,R102K,D106E"), H1 correspond to amino acids 26-35 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Ala is substituted for Ser at amino acid position 31, H2 corresponds to amino acids 50-66 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Ala is substituted for Ser at amino acid position 54, and H3 corresponds to amino acids 99-111 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Glu is substituted for Asp at amino acid position 100, Lys is substituted for Arg at amino acid position 102 and Glu is substituted for Asp at amino acid position 106.

As in the humanization of the light chain variable domain described above, a humanized heavy chain variable domain is constructed by substituting the CDRs of 6G4.2.5HV or the CDRs of a 6G4.2.5HV CDRs variant for the corresponding sequences in a human heavy chain variable domain. The humanized heavy chain variable domain comprising the CDRs of 6G4.2.5HV or the CDRs of a 6G4.2.5HV CDRs variant can also contain some FR residues that are substituted by residues from analogous sites in the murine 6G4.2.5 antibody heavy chain variable domain. The complete amino acid sequence of 6G4.2.5HV is set out as amino acids 1-122 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50).

The choice of human variable domains, both light and heavy, to be used in making the humanized antibodies and antibody fragments is very important to reduce antigenicity. According to the so-called "best-fit" method, the sequence of the variable domain of a rodent antibody is screened against the entire library of known human variable-domain sequences. The human sequence which is closest to that of the rodent is then accepted as the human framework (FR) for the humanized antibody (Sims *et al.*, J. Immunol. 151: 2296 (1993); Chothia and Lesk, J. Mol. Biol. 196:901 (1987)). Another method uses a particular framework derived from the consensus sequence of all human antibodies of a particular subgroup of light or heavy chains. The same framework can be used for several different humanized antibodies (Carter *et al.*, Proc. Natl. Acad. Sci. U.S.A. 89:4285 (1992); Presta *et al.*, J. Immunol. 151:2623 (1993)).

It is also important that the antibodies and antibody fragments of the invention be humanized with retention of high affinity for human IL-8 and other favorable biological properties. To achieve this goal, according to a preferred method, the humanized antibodies and antibody fragments of the invention are prepared by a process of analysis of the parental sequences and various conceptual humanized products using three-dimensional models of the parental and humanized sequences. Three-dimensional immunoglobulin models are commonly available and are familiar to those skilled in the art. Computer programs are available which illustrate and display probable three-dimensional conformational structures of selected candidate immunoglobulin sequences. Inspection of these displays permits analysis of the likely

role of the residues in the functioning of the candidate immunoglobulin sequence, i.e., the analysis of residues that influence the ability of the candidate immunoglobulin to bind its antigen. In this way, FR residues can be selected and combined from the consensus and parental sequences so that the desired antibody characteristic, such as increased affinity for the target antigen(s), is achieved.

5 Any and all humanized light chain variable domain amino acid sequences which comprise the CDRs of 6G4.2.5LV are collectively referred to herein as "hu6G4.2.5LV".

Any and all humanized light chain variable domain amino acid sequences which comprise the CDRs of 6G4.2.5LV/L1N35X₃₅ are collectively referred to herein as "hu 6G4.2.5LV/L1N35X₃₅".

10 Any and all humanized light chain variable domain amino acid sequences which comprise the CDRs of 6G4.2.5LV/L1N35A are collectively referred to herein as "hu6G4.2.5LV/L1N35A".

Any and all humanized light chain variable domain amino acid sequences which comprise the CDRs of 6G4.2.5LV/L1N35E are collectively referred to herein as "hu6G4.2.5LV/L1N35E".

Any and all humanized light chain variable domain amino acid sequences which comprise the CDRs of 6G4.2.5LV/L1S26X₂₆ are collectively referred to herein as "hu6G4.2.5LV/L1S26X₂₆".

15 Any and all humanized light chain variable domain amino acid sequences which comprise the CDRs of 6G4.2.5LV/L1S26A are collectively referred to herein as "hu6G4.2.5LV/L1S26A".

Any and all humanized light chain variable domain amino acid sequences which comprise the CDRs of 6G4.2.5LV/L3H98X₉₈ are collectively referred to herein as "hu6G4.2.5LV/L3H98X₉₈".

20 Any and all humanized light chain variable domain amino acid sequences which comprise the CDRs of 6G4.2.5LV/L3H98A are collectively referred to herein as "hu6G4.2.5LV/L3H98A".

Any and all humanized light chain variable domain amino acid sequences which comprise the CDRs of 6G4.2.5LV/L1S26X₂₆,N35X₃₅ are collectively referred to herein as "hu6G4.2.5LV/L1S26X₂₆,N35X₃₅".

25 Any and all humanized light chain variable domain amino acid sequences which comprise the CDRs of 6G4.2.5LV/L1S26A,N35A are collectively referred to herein as "hu6G4.2.5LV/L1S26A,N35A".

Any and all humanized light chain variable domain amino acid sequences which comprise the CDRs of 6G4.2.5LV/L1N35X₃₅/L3H98X₉₈ are collectively referred to herein as "hu6G4.2.5LV/L1N35X₃₅/L3H98X₉₈".

30 Any and all humanized light chain variable domain amino acid sequences which comprise the CDRs of 6G4.2.5LV/L1N35A/L3H98A are collectively referred to herein as "hu6G4.2.5LV/L1N35A/L3H98A".

Any and all humanized light chain variable domain amino acid sequences which comprise the CDRs of 6G4.2.5LV/L1S26X₂₆/L3H98X₉₈ are collectively referred to herein as "hu6G4.2.5LV/L1S26X₂₆/L3H98X₉₈".

Any and all humanized light chain variable domain amino acid sequences which comprise the CDRs of 6G4.2.5LV/L1S26A/L3H98A are collectively referred to herein as "hu6G4.2.5LV/L1S26A/L3H98A".

Any and all humanized light chain variable domain amino acid sequences which comprise the CDRs of 6G4.2.5LV/L1S26X₂₆,N35X₃₅/L3H98X₉₈ are collectively referred to herein as "hu6G4.2.5LV/L1S26X₂₆,N35X₃₅/L3H98X₉₈".

Any and all humanized light chain variable domain amino acid sequences which comprise the CDRs of 6G4.2.5LV/L1S26A,N35A/L3H98A are collectively referred to herein as "hu6G4.2.5LV/L1S26A,N35A/L3H98A".

10 The humanized light chain variable domain amino acid sequences of hu6G4.2.5LV/L1N35X₃₅, hu6G4.2.5LV/L1S26X₂₆, hu6G4.2.5LV/L1S26X₂₆/L3H98X₉₈, hu6G4.2.5LV/L1S26X₂₆,N35X₃₅, hu6G4.2.5LV/L1N35X₃₅/L3H98X₉₈, hu6G4.2.5LV/L1S26X₂₆/L3H98X₉₈, and hu6G4.2.5LV/L1S26X₂₆,N35X₃₅/L3H98X₉₈ are collectively referred to herein as "hu6G4.2.5LV/vL1-3X".

15 The humanized light chain variable domain amino acid sequences of hu6G4.2.5LV/L1N35A, hu6G4.2.5LV/L1S26A, hu6G4.2.5LV/L1S26A/L3H98A, hu6G4.2.5LV/L1S26A,N35A, hu6G4.2.5LV/L1N35A/L3H98A, hu6G4.2.5LV/L1S26A/L3H98A, hu6G4.2.5LV/L1S26A,N35A/L3H98A are collectively referred to herein as "hu6G4.2.5LV/vL1-3A".

Any and all humanized heavy chain variable domain amino acid sequences which comprise the CDRs of 6G4.2.5HV are collectively referred to herein as "hu6G4.2.5HV".

20 Any and all humanized heavy chain variable domain amino acid sequences which comprise the CDRs of 6G4.2.5HV/H1S31Z₃₁ are collectively referred to herein as "hu6G4.2.5HV/H1S31Z₃₁".

Any and all humanized heavy chain variable domain amino acid sequences which comprise the CDRs of 6G4.2.5HV/H1S31A are collectively referred to herein as "hu6G4.2.5HV/H1S31A".

25 Any and all humanized heavy chain variable domain amino acid sequences which comprise the CDRs of 6G4.2.5HV/H2S54Z₅₄ are collectively referred to herein as "hu6G4.2.5HV/H2S54Z₅₄".

Any and all humanized heavy chain variable domain amino acid sequences which comprise the CDRs of 6G4.2.5HV/H2S54A are collectively referred to herein as "hu6G4.2.5HV/H2S54A".

Any and all humanized heavy chain variable domain amino acid sequences which comprise the CDRs of 6G4.2.5HV/H3D100E are collectively referred to herein as "hu6G4.2.5HV/H3D100E".

30 Any and all humanized heavy chain variable domain amino acid sequences which comprise the CDRs of 6G4.2.5HV/H3R102K are collectively referred to herein as "hu6G4.2.5HV/H3R102K".

Any and all humanized heavy chain variable domain amino acid sequences which comprise the CDRs of 6G4.2.5HV/H3D106E are collectively referred to herein as "hu6G4.2.5HV/H3D106E".

Any and all humanized heavy chain variable domain amino acid sequences which comprise the

CDRs of 6G4.2.5HV/H3D100E,R102K are collectively referred to herein as "hu6G4.2.5HV/H3D100E,R102K".

Any and all humanized heavy chain variable domain amino acid sequences which comprise the CDRs of 6G4.2.5HV/H3R102K,D106E are collectively referred to herein as

5 "hu6G4.2.5HV/H3R102K,D106E".

Any and all humanized heavy chain variable domain amino acid sequences which comprise the CDRs of 6G4.2.5HV/H3D100E,D106E are collectively referred to herein as "hu6G4.2.5HV/H3D100E,D106E".

10 Any and all humanized heavy chain variable domain amino acid sequences which comprise the CDRs of 6G4.2.5HV/H3D100E,R102K,D106E are collectively referred to herein as "hu6G4.2.5HV/H3D100E,R102K,D106E".

Any and all humanized heavy chain variable domain amino acid sequences which comprise the CDRs of 6G4.2.5HV/H1S31Z₃₁/H2S54Z₅₄ are collectively referred to herein as

"hu6G4.2.5HV/H1S31Z₃₁/H2S54Z₅₄".

15 Any and all humanized heavy chain variable domain amino acid sequences which comprise the CDRs of 6G4.2.5HV/H1S31Z₃₁/H3D100E are collectively referred to herein as

"hu6G4.2.5HV/H1S31Z₃₁/H3D100E".

~~Any and all humanized heavy chain variable domain amino acid sequences which comprise the~~
CDRs of 6G4.2.5HV/H1S31Z₃₁/H3R102K are collectively referred to herein as

20 "hu6G4.2.5HV/H1S31Z₃₁/H3R102K".

Any and all humanized heavy chain variable domain amino acid sequences which comprise the CDRs of 6G4.2.5HV/H1S31Z₃₁/H3D106E are collectively referred to herein as

"hu6G4.2.5HV/H1S31Z₃₁/H3D106E".

25 Any and all humanized heavy chain variable domain amino acid sequences which comprise the CDRs of 6G4.2.5HV/H1S31Z₃₁/H3D100E,R102K are collectively referred to herein as

"hu6G4.2.5HV/H1S31Z₃₁/H3D100E,R102K".

Any and all humanized heavy chain variable domain amino acid sequences which comprise the CDRs of 6G4.2.5HV/H1S31Z₃₁/H3R102K,D106E are collectively referred to herein as

"hu6G4.2.5HV/H1S31Z₃₁/H3R102K,D106E".

30 Any and all humanized heavy chain variable domain amino acid sequences which comprise the CDRs of 6G4.2.5HV/H1S31Z₃₁/H3D100E,D106E are collectively referred to herein as

"hu6G4.2.5HV/H1S31Z₃₁/H3D100E,D106E".

Any and all humanized heavy chain variable domain amino acid sequences which comprise the CDRs of 6G4.2.5HV/H1S31Z₃₁/H3D100E,R102K,D106E are collectively referred to herein as "hu6G4.2.5HV/H1S31Z₃₁/H3D100E,R102K,D106E".

Any and all humanized heavy chain variable domain amino acid sequences which comprise the CDRs of 6G4.2.5HV/H2S54Z₅₄/H3D100E are collectively referred to herein as "hu6G4.2.5HV/H2S54Z₅₄/H3D100E".

Any and all humanized heavy chain variable domain amino acid sequences which comprise the CDRs of 6G4.2.5HV/H2S54Z₅₄/H3R102K are collectively referred to herein as "hu6G4.2.5HV/H2S54Z₅₄/H3R102K".

Any and all humanized heavy chain variable domain amino acid sequences which comprise the CDRs of 6G4.2.5HV/H2S54Z₅₄/H3D106E are collectively referred to herein as "hu6G4.2.5HV/H2S54Z₅₄/H3D106E".

Any and all humanized heavy chain variable domain amino acid sequences which comprise the CDRs of 6G4.2.5HV/H2S54Z₅₄/H3R102K,D106E are collectively referred to herein as "hu6G4.2.5HV/H2S54Z₅₄/H3R102K,D106E".

Any and all humanized heavy chain variable domain amino acid sequences which comprise the CDRs of 6G4.2.5HV/H2S54Z₅₄/H3D100E,D106E are collectively referred to herein as "hu6G4.2.5HV/H2S54Z₅₄/H3D100E,D106E".

Any and all humanized heavy chain variable domain amino acid sequences which comprise the CDRs of 6G4.2.5HV/H2S54Z₅₄/H3D100E,R102K,D106E are collectively referred to herein as "hu6G4.2.5HV/H2S54Z₅₄/H3D100E,R102K,D106E".

Any and all humanized heavy chain variable domain amino acid sequences which comprise the CDRs of 6G4.2.5HV/H1S31Z₃₁/H2S54Z₅₄/H3D100E are collectively referred to herein as "hu6G4.2.5HV/H1S31Z₃₁/H2S54Z₅₄/H3D100E".

Any and all humanized heavy chain variable domain amino acid sequences which comprise the CDRs of 6G4.2.5HV/H1S31Z₃₁/H2S54Z₅₄/H3R102K are collectively referred to herein as "hu6G4.2.5HV/H1S31Z₃₁/H2S54Z₅₄/H3R102K".

Any and all humanized heavy chain variable domain amino acid sequences which comprise the CDRs of 6G4.2.5HV/H1S31Z₃₁/H2S54Z₅₄/H3D106E are collectively referred to herein as "hu6G4.2.5HV/H1S31Z₃₁/H2S54Z₅₄/H3D106E".

Any and all humanized heavy chain variable domain amino acid sequences which comprise the CDRs of 6G4.2.5HV/HIS31Z₃₁/H2S54Z₅₄/H3D100E,R102K are collectively referred to herein as "hu6G4.2.5HV/HIS31Z₃₁/H2S54Z₅₄/H3D100E,R102K".

Any and all humanized heavy chain variable domain amino acid sequences which comprise the CDRs of 6G4.2.5HV/HIS31Z₃₁/H2S54Z₅₄/H3R102K,D106E are collectively referred to herein as "hu6G4.2.5HV/HIS31Z₃₁/H2S54Z₅₄/H3R102K,D106E".

Any and all humanized heavy chain variable domain amino acid sequences which comprise the CDRs of 6G4.2.5HV/HIS31Z₃₁/H2S54Z₅₄/H3D100E,D106E are collectively referred to herein as "hu6G4.2.5HV/HIS31Z₃₁/H2S54Z₅₄/H3D100E,D106E".

Any and all humanized heavy chain variable domain amino acid sequences which comprise the CDRs of 6G4.2.5HV/HIS31Z₃₁/H2S54Z₅₄/H3D100E,R102K,D106E are collectively referred to herein as "hu6G4.2.5HV/HIS31Z₃₁/H2S54Z₅₄/H3D100E,R102K,D106E".

Any and all humanized heavy chain variable domain amino acid sequences which comprise the CDRs of 6G4.2.5HV/HIS31A/H2S54A are collectively referred to herein as "hu6G4.2.5HV/HIS31A/H2S54A".

Any and all humanized heavy chain variable domain amino acid sequences which comprise the CDRs of 6G4.2.5HV/HIS31A/H3D100E are collectively referred to herein as "hu6G4.2.5HV/HIS31A/H3D100E".

Any and all humanized heavy chain variable domain amino acid sequences which comprise the CDRs of 6G4.2.5HV/HIS31A/H3R102K are collectively referred to herein as "hu6G4.2.5HV/HIS31A/H3R102K".

Any and all humanized heavy chain variable domain amino acid sequences which comprise the CDRs of 6G4.2.5HV/HIS31A/H3D106E are collectively referred to herein as "hu6G4.2.5HV/HIS31A/H3D106E".

Any and all humanized heavy chain variable domain amino acid sequences which comprise the CDRs of 6G4.2.5HV/HIS31A/H3D100E,R102K are collectively referred to herein as "hu6G4.2.5HV/HIS31A/H3D100E,R102K".

Any and all humanized heavy chain variable domain amino acid sequences which comprise the CDRs of 6G4.2.5HV/HIS31A/H3R102K,D106E are collectively referred to herein as "hu6G4.2.5HV/HIS31A/H3R102K,D106E".

Any and all humanized heavy chain variable domain amino acid sequences which comprise the CDRs of 6G4.2.5HV/HIS31A/H3D100E,D106E are collectively referred to herein as "hu6G4.2.5HV/HIS31A/H3D100E,D106E".

Any and all humanized heavy chain variable domain amino acid sequences which comprise the

CDRs of 6G4.2.5HV/H1S31A/H3D100E,R102K,D106E are collectively referred to herein as "hu6G4.2.5HV/H1S31A/H3D100E,R102K,D106E".

Any and all humanized heavy chain variable domain amino acid sequences which comprise the CDRs of 6G4.2.5HV/H2S54A/H3D100E are collectively referred to herein as
5 "hu6G4.2.5HV/H2S54A/H3D100E".

Any and all humanized heavy chain variable domain amino acid sequences which comprise the CDRs of 6G4.2.5HV/H2S54A/H3R102K are collectively referred to herein as
"hu6G4.2.5HV/H2S54A/H3R102K".

Any and all humanized heavy chain variable domain amino acid sequences which comprise the CDRs of 6G4.2.5HV/H2S54A/H3D106E are collectively referred to herein as
10 "hu6G4.2.5HV/H2S54A/H3D106E".

Any and all humanized heavy chain variable domain amino acid sequences which comprise the CDRs of 6G4.2.5HV/H2S54A/H3R102K,D106E are collectively referred to herein as
"hu6G4.2.5HV/H2S54A/H3R102K,D106E".

Any and all humanized heavy chain variable domain amino acid sequences which comprise the CDRs of 6G4.2.5HV/H2S54A/H3D100E,D106E are collectively referred to herein as
15 "hu6G4.2.5HV/H2S54A/H3D100E,D106E".

Any and all humanized heavy chain variable domain amino acid sequences which comprise the CDRs of 6G4.2.5HV/H2S54A/H3D100E,R102K,D106E are collectively referred to herein as
20 "hu6G4.2.5HV/H2S54A/H3D100E,R102K,D106E".

Any and all humanized heavy chain variable domain amino acid sequences which comprise the CDRs of 6G4.2.5HV/H1S31A/H2S54A/H3D100E are collectively referred to herein as
"hu6G4.2.5HV/H1S31A/H2S54A/H3D100E".

Any and all humanized heavy chain variable domain amino acid sequences which comprise the CDRs of 6G4.2.5HV/H1S31A/H2S54A/H3R102K are collectively referred to herein as
25 "hu6G4.2.5HV/H1S31A/H2S54A/H3R102K".

Any and all humanized heavy chain variable domain amino acid sequences which comprise the CDRs of 6G4.2.5HV/H1S31A/H2S54A/H3D106E are collectively referred to herein as
"hu6G4.2.5HV/H1S31A/H2S54A/H3D106E".

Any and all humanized heavy chain variable domain amino acid sequences which comprise the CDRs of 6G4.2.5HV/H1S31A/H2S54A/H3D100E,R102K are collectively referred to herein as
30 "hu6G4.2.5HV/H1S31A/H2S54A/H3D100E,R102K".

Any and all humanized heavy chain variable domain amino acid sequences which comprise the CDRs of 6G4.2.5HV/H1S31A/H2S54A/H3R102K,D106E are collectively referred to herein as
35 "hu6G4.2.5HV/H1S31A/H2S54A/H3R102K,D106E".

Any and all humanized heavy chain variable domain amino acid sequences which comprise the CDRs of 6G4.2.5HV/H1S31A/H2S54A/H3D100E,D106E are collectively referred to herein as

"hu6G4.2.5HV/H1S31A/H2S54A/H3D100E,D106E".

Any and all humanized heavy chain variable domain amino acid sequences which comprise the CDRs of 6G4.2.5HV/H1S31A/H2S54A/H3D100E,R102K,D106E are collectively referred to herein as "hu6G4.2.5HV/H1S31A/H2S54A/H3D100E,R102K,D106E".

- 5 The humanized heavy chain variable domain amino acid sequences of
 hu6G4.2.5HV/H1S31Z₃₁, hu6G4.2.5HV/H2S54Z₅₄, hu6G4.2.5HV/H3D100E, hu6G4.2.5HV/H3R102K,
 hu6G4.2.5HV/H3D106E, hu6G4.2.5HV/H3D100E,R102K, hu6G4.2.5HV/H3R102K,D106E,
 hu6G4.2.5HV/H3D100E,D106E, hu6G4.2.5HV/H3D100E,R102K,D106E,
 hu6G4.2.5HV/H1S31Z₃₁/H2S54Z₅₄, hu6G4.2.5HV/H1S31Z₃₁/H3D100E,
 10 hu6G4.2.5HV/H1S31Z₃₁/H3R102K, hu6G4.2.5HV/H1S31Z₃₁/H3D106E,
 hu6G4.2.5HV/H1S31Z₃₁/H3D100E,R102K, hu6G4.2.5HV/H1S31Z₃₁/H3R102K,D106E,
 hu6G4.2.5HV/H1S31Z₃₁/H3D100E,D106E, hu6G4.2.5HV/H1S31Z₃₁/H3D100E,R102K,D106E,
 hu6G4.2.5HV/H2S54Z₅₄/H3D100E, hu6G4.2.5HV/H2S54Z₅₄/H3R102K,
 hu6G4.2.5HV/H2S54Z₅₄/H3D106E, hu6G4.2.5HV/H2S54Z₅₄/H3R102K,D106E, hu6G4.2.5HV/H2S54Z
 15 ₅₄/H3D100E,D106E, hu6G4.2.5HV/H2S54Z₅₄/H3D100E,R102K,D106E,
 hu6G4.2.5HV/H1S31Z₃₁/H2S54Z₅₄/H3D100E, hu6G4.2.5HV/H1S31Z₃₁/H2S54Z₅₄/H3R102K,
 hu6G4.2.5HV/H1S31Z₃₁/H2S54Z₅₄/H3D106E, hu6G4.2.5HV/H1S31Z₃₁/H2S54Z₅₄/H3D100E,R102K,
 hu6G4.2.5HV/H1S31Z₃₁/H2S54Z₅₄/H3R102K,D106E,
 hu6G4.2.5HV/H1S31Z₃₁/H2S54Z₅₄/H3D100E,D106E, and hu6G4.2.5HV/H1S31Z₃₁/H2S54Z
 20 ₅₄/H3D100E,R102K,D106E are collectively referred to herein as "hu6G4.2.5HV/vH1-3Z".

- The humanized heavy chain variable domain amino acid sequences of
 hu6G4.2.5HV/H1S31A, hu6G4.2.5HV/H2S54A, hu6G4.2.5HV/H3D100E, hu6G4.2.5HV/H3R102K,
 hu6G4.2.5HV/H3D106E, hu6G4.2.5HV/H3D100E,R102K, hu6G4.2.5HV/H3R102K,D106E,
 hu6G4.2.5HV/H3D100E,D106E, hu6G4.2.5HV/H3D100E,R102K,D106E,
 25 hu6G4.2.5HV/H1S31A/H2S54A, hu6G4.2.5HV/H1S31A/H3D100E, hu6G4.2.5HV/H1S31A/H3R102K,
 hu6G4.2.5HV/H1S31A/H3D106E, hu6G4.2.5HV/H1S31A/H3D100E,R102K,
 hu6G4.2.5HV/H1S31A/H3R102K,D106E, hu6G4.2.5HV/H1S31A/H3D100E,D106E,
 hu6G4.2.5HV/H1S31A/H3D100E,R102K,D106E, hu6G4.2.5HV/H2S54A/H3D100E,
 hu6G4.2.5HV/H2S54A/H3R102K, hu6G4.2.5HV/H2S54A/H3D106E,
 30 hu6G4.2.5HV/H2S54A/H3R102K,D106E, hu6G4.2.5HV/H2S54A/H3D100E,D106E,
 hu6G4.2.5HV/H2S54A/H3D100E,R102K,D106E, hu6G4.2.5HV/H1S31A/H2S54A/H3D100E,
 hu6G4.2.5HV/H1S31A/H2S54A/H3R102K, hu6G4.2.5HV/H1S31A/H2S54A/H3D106E,

hu6G4.2.5HV/H1S31A/H2S54A/H3D100E,R102K, hu6G4.2.5HV/H1S31A/H2S54A/H3R102K,D106E, hu6G4.2.5HV/H1S31A/H2S54A/H3D100E,D106E, and hu6G4.2.5HV/H1S31A/H2S54A/H3D100E,R102K,D106E are collectively referred to herein as "hu6G4.2.5HV/vH1-3A".

- 5 The invention provides a humanized antibody or antibody fragment that comprises a light chain variable domain comprising the hu6G4.2.5LV/vL1-3X. In another embodiment, the invention provides a humanized antibody or antibody fragment that comprises a light chain variable domain comprising the hu6G4.2.5LV/vL1-3A. In yet another embodiment, the invention provides a humanized antibody or antibody fragment that comprises a light chain variable domain comprising the hu6G4.2.5LV/L1N35X₃₅.
- 10 In still another embodiment, the invention provides a humanized antibody or antibody fragment that comprises a light chain variable domain comprising the hu6G4.2.5LV/L1N35A. In a further embodiment, the invention provides a humanized antibody or antibody fragment that comprises a light chain variable domain comprising the hu6G4.2.5LV/L1N35E.

- 15 The invention additionally provides a humanized antibody or antibody fragment that comprises a light chain variable domain comprising the hu6G4.2.5LV/vL1-3X, and further comprises a heavy chain variable domain comprising the hu6G4.2.5HV or hu6G4.2.5HV/vH1-3Z. In another embodiment, the invention provides a humanized antibody or antibody fragment that comprises a light chain variable domain comprising the hu6G4.2.5LV/vL1-3A, and further comprises a heavy chain variable domain comprising the hu6G4.2.5HV or hu6G4.2.5HV/vH1-3Z. In yet another embodiment, the invention provides a humanized antibody or antibody fragment that comprises a light chain variable domain comprising the hu6G4.2.5LV/vL1-3A, and further comprises a heavy chain variable domain comprising the hu6G4.2.5HV/vH1-3A.
- 20 In a further embodiment, the invention provides a humanized antibody or antibody fragment that comprises a light chain variable domain comprising the hu6G4.2.5LV/L1N35X₃₅, and further comprises a

- 25 heavy chain variable domain comprising the hu6G4.2.5HV or hu6G4.2.5HV/vH1-3Z. In another embodiment, the invention provides a humanized antibody or antibody fragment that comprises a light chain variable domain comprising the hu6G4.2.5LV/N35X₃₅, and further comprises a heavy chain variable domain comprising the hu6G4.2.5HV/vH1-3A. In a preferred embodiment, the antibody or antibody fragment comprises a light chain variable domain comprising the hu6G4.2.5LV/L1N35X₃₅ and further
- 30 comprises a humanized heavy chain comprising the amino acid sequence of 6G4.2.5HV11.

- 35 In an additional embodiment, the invention provides a humanized antibody or antibody fragment that comprises a light chain variable domain comprising the hu6G4.2.5LV/L1N35A, and further comprises a heavy chain variable domain comprising the hu6G4.2.5HV or hu6G4.2.5HV/vH1-3Z. In another embodiment, the invention provides a humanized antibody or antibody fragment that comprises a light chain variable domain comprising the hu6G4.2.5LV/N35A, and further comprises a heavy chain variable domain comprising the hu6G4.2.5HV/vH1-3A. In still another embodiment, the humanized antibody or antibody

fragment comprises a light chain variable domain comprising the hu6G4.2.5LV/LIN35A, and further comprises a heavy chain variable domain comprising the hu6G4.2.5HV. In a further embodiment, the humanized antibody or antibody fragment comprises a light chain variable domain comprising the hu6G4.2.5LV/LIN35E, and further comprises a heavy chain variable domain comprising the hu6G4.2.5HV.

5 In a preferred embodiment, the antibody or antibody fragment comprises a light chain variable domain comprising the hu6G4.2.5LV/LIN35A and further comprises a humanized heavy chain comprising the amino acid sequence of 6G4.2.5HV11. In another preferred embodiment, the antibody or antibody fragment comprises a light chain variable domain comprising the hu6G4.2.5LV/LIN35E and further comprises a humanized heavy chain comprising the amino acid sequence of 6G4.2.5HV11.

10 The invention encompasses a single chain antibody fragment comprising the hu6G4.2.5LV/vL1-3X, with or without any additional amino acid sequence. In one embodiment, the invention provides a single chain antibody fragment comprising the hu6G4.2.5LV/vL1-3X without any associated heavy chain variable domain amino acid sequence, i.e. a single chain species that makes up one half of an Fv fragment. In another embodiment, the invention provides a single chain antibody fragment comprising the
15 hu6G4.2.5LV/vL1-3A without any associated heavy chain variable domain amino acid sequence. In still another embodiment, the invention provides a single chain antibody fragment comprising the hu6G4.2.5LV/LIN35X₃₅ without any associated heavy chain variable domain amino acid sequence. In a preferred embodiment, the invention provides a single chain antibody fragment comprising the hu6G4.2.5LV/LIN35A without any associated heavy chain variable domain amino acid sequence. In
20 another preferred embodiment, the invention provides a single chain antibody fragment comprising the hu6G4.2.5LV/LIN35E without any associated heavy chain variable domain amino acid sequence.

In one embodiment, the invention provides a single chain antibody fragment wherein the hu6G4.2.5LV/vL1-3X and the hu6G4.2.5HV or hu6G4.2.5HV/vH1-3Z are contained in a single chain polypeptide species. In a preferred embodiment, the single chain antibody fragment is a scFv species
25 comprising the hu6G4.2.5LV/vL1-3X joined to the hu6G4.2.5HV or hu6G4.2.5HV/vH1-3Z by means of a flexible peptide linker sequence, wherein the heavy chain and light chain variable domains can associate in a "dimeric" structure analogous to that formed in a two-chain Fv species. In another embodiment, the single chain antibody fragment is a species comprising the hu6G4.2.5LV/vL1-3X joined to the hu6G4.2.5HV or hu6G4.2.5HV/vH1-3Z by a linker that is too short to permit intramolecular pairing of the two variable
30 domains, i.e. a single chain polypeptide monomer that forms a diabody upon dimerization with another monomer.

In another embodiment, the invention provides a single chain antibody fragment wherein the hu6G4.2.5LV/vL1-3A and the hu6G4.2.5HV or hu6G4.2.5HV/vH1-3Z are contained in a single chain polypeptide species. In a preferred embodiment, the single chain antibody fragment is a scFv species
35 comprising the hu6G4.2.5LV/vL1-3A joined to the hu6G4.2.5HV or hu6G4.2.5HV/vH1-3Z by means of a flexible peptide linker sequence, wherein the heavy chain and light chain variable domains can associate in a "dimeric" structure analogous to that formed in a two-chain Fv species. In another embodiment, the single

chain antibody fragment is a species comprising the hu6G4.2.5LV/vL1-3A joined to the hu6G4.2.5HV or hu6G4.2.5HV/vH1-3Z by a linker that is too short to permit intramolecular pairing of the two variable domains, i.e. a single chain polypeptide monomer that forms a diabody upon dimerization with another monomer.

5 In yet another embodiment, the invention provides a single chain antibody fragment wherein the hu6G4.2.5LV/vL1-3A and the hu6G4.2.5HV/vH1-3A are contained in a single-chain-polypeptide species. In a preferred embodiment, the single chain antibody fragment is a scFv species comprising the hu6G4.2.5LV/vL1-3A joined to the hu6G4.2.5HV/vH1-3A by means of a flexible peptide linker sequence, wherein the heavy chain and light chain variable domains can associate in a "dimeric" structure analogous to
10 that formed in a two-chain Fv species. In another embodiment, the single chain antibody fragment is a species comprising the hu6G4.2.5LV/vL1-3A joined to the hu6G4.2.5HV/vH1-3A by a linker that is too short to permit intramolecular pairing of the two variable domains, i.e. a single chain polypeptide monomer that forms a diabody upon dimerization with another monomer.

In still another embodiment, the invention provides a single chain antibody fragment wherein the
15 hu6G4.2.5LV/L1N35X₃₅ and the hu6G4.2.5HV or hu6G4.2.5HV/vH1-3Z are contained in a single chain polypeptide species. In a preferred embodiment, the single chain antibody fragment is a scFv species comprising the hu6G4.2.5LV/L1N35X₃₅ joined to the hu6G4.2.5HV or hu6G4.2.5HV/vH1-3Z by means of a flexible peptide linker sequence, wherein the heavy chain and light chain variable domains can associate in a "dimeric" structure analogous to that formed in a two-chain Fv species. In another embodiment, the single
20 chain antibody fragment is a species comprising the hu6G4.2.5LV/L1N35X₃₅ joined to the hu6G4.2.5HV or hu6G4.2.5HV/vH1-3Z by a linker that is too short to permit intramolecular pairing of the two variable domains, i.e. a single chain polypeptide monomer that forms a diabody upon dimerization with another monomer.

In a further embodiment, the invention provides a single chain antibody fragment wherein the
25 hu6G4.2.5LV/L1N35X₃₅ and the hu6G4.2.5HV/vH1-3A are contained in a single chain polypeptide species. In a preferred embodiment, the single chain antibody fragment is a scFv species comprising the hu6G4.2.5LV/L1N35X₃₅ joined to the hu6G4.2.5HV/vH1-3A by means of a flexible peptide linker sequence, wherein the heavy chain and light chain variable domains can associate in a "dimeric" structure analogous to that formed in a two-chain Fv species. In another embodiment, the single chain antibody
30 fragment is a species comprising the hu6G4.2.5LV/L1N35X₃₅ joined to the hu6G4.2.5HV/vH1-3A by a linker that is too short to permit intramolecular pairing of the two variable domains, i.e. a single chain polypeptide monomer that forms a diabody upon dimerization with another monomer.

In an additional embodiment, the invention provides a single chain antibody fragment wherein the
35 hu6G4.2.5LV/L1N35A and the hu6G4.2.5HV or hu6G4.2.5HV/vH1-3Z are contained in a single chain polypeptide species. In a preferred embodiment, the single chain antibody fragment is a scFv species

comprising the hu6G4.2.5LV/L1N35A joined to the hu6G4.2.5HV or hu6G4.2.5HV/vH1-3Z by means of a flexible peptide linker sequence, wherein the heavy chain and light chain variable domains can associate in a "dimeric" structure analogous to that formed in a two-chain Fv species. In another embodiment, the single chain antibody fragment is a species comprising the hu6G4.2.5LV/L1N35A joined to the hu6G4.2.5HV or hu6G4.2.5HV/vH1-3Z by a linker that is too short to permit intramolecular pairing of the two variable domains, i.e. a single chain polypeptide monomer that forms a diabody upon dimerization with another monomer.

Also provided herein is a single chain antibody fragment wherein the hu6G4.2.5LV/L1N35E and the hu6G4.2.5HV are contained in a single chain polypeptide species. In a preferred embodiment, the single chain antibody fragment is a scFv species comprising the hu6G4.2.5LV/L1N35E joined to the hu6G4.2.5HV by means of a flexible peptide linker sequence, wherein the heavy chain and light chain variable domains can associate in a "dimeric" structure analogous to that formed in a two-chain Fv species. In another embodiment, the single chain antibody fragment is a species comprising the hu6G4.2.5LV/L1N35E joined to the hu6G4.2.5HV by a linker that is too short to permit intramolecular pairing of the two variable domains, i.e. a single chain polypeptide monomer that forms a diabody upon dimerization with another monomer.

In still another embodiment, the invention provides a single chain antibody fragment wherein the hu6G4.2.5LV/L1N35A and the hu6G4.2.5HV/vH1-3A are contained in a single chain polypeptide species. In a preferred embodiment, the single chain antibody fragment is a scFv species comprising the hu6G4.2.5LV/L1N35A joined to the hu6G4.2.5HV/vH1-3A by means of a flexible peptide linker sequence, wherein the heavy chain and light chain variable domains can associate in a "dimeric" structure analogous to that formed in a two-chain Fv species. In another embodiment, the single chain antibody fragment is a species comprising the hu6G4.2.5LV/L1N35A joined to the hu6G4.2.5HV/vH1-3A by a linker that is too short to permit intramolecular pairing of the two variable domains, i.e. a single chain polypeptide monomer that forms a diabody upon dimerization with another monomer.

In yet another embodiment, the invention provides an antibody fragment comprising a plurality of polypeptide chains, wherein one polypeptide chain comprises the hu6G4.2.5LV/vL1-3X and a second polypeptide chain comprises the hu6G4.2.5HV or hu6G4.2.5HV/vH1-3Z and the two polypeptide chains are covalently linked by one or more interchain disulfide bonds.

In still another embodiment, the invention provides an antibody fragment comprising a plurality of polypeptide chains, wherein one polypeptide chain comprises the hu6G4.2.5LV/vL1-3X and a second polypeptide chain comprises the hu6G4.2.5HV/vH1-3A and the two polypeptide chains are covalently linked by one or more interchain disulfide bonds. In a preferred embodiment, the invention provides an antibody fragment comprising a plurality of polypeptide chains, wherein one polypeptide chain comprises the hu6G4.2.5LV/vL1-3X and a second polypeptide chain comprises the amino acid sequence of hu6G4.2.5HV11 and the two polypeptide chains are covalently linked by one or more interchain disulfide bonds.

In a further embodiment, the invention provides an antibody fragment comprising a plurality of polypeptide chains, wherein one polypeptide chain comprises the hu6G4.2.5LV/vL1-3A and a second polypeptide chain comprises the hu6G4.2.5HV or hu6G4.2.5HV/vH1-3Z and the two polypeptide chains are covalently linked by one or more interchain disulfide bonds.

5 In still another embodiment, the invention provides an antibody fragment comprising a plurality of polypeptide chains, wherein one polypeptide chain comprises the hu6G4.2.5LV/vL1-3A and a second polypeptide chain comprises the hu6G4.2.5HV/vH1-3A and the two polypeptide chains are covalently linked by one or more interchain disulfide bonds. In a preferred embodiment, the invention provides an antibody fragment comprising a plurality of polypeptide chains, wherein one polypeptide chain comprises
10 the hu6G4.2.5LV/vL1-3A and a second polypeptide chain comprises the amino acid sequence of 6G4.2.5HV11 and the two polypeptide chains are covalently linked by one or more interchain disulfide bonds.

The invention also encompasses an antibody fragment comprising a plurality of polypeptide chains, wherein one polypeptide chain comprises the hu6G4.2.5LV/L1N35X₃₅ and a second polypeptide chain
15 comprises the hu6G4.2.5HV or hu6G4.2.5HV/vH1-3Z and the two polypeptide chains are covalently linked by one or more interchain disulfide bonds.

In still another embodiment, the invention provides an antibody fragment comprising a plurality of polypeptide chains, wherein one polypeptide chain comprises the hu6G4.2.5LV/L1N35X₃₅ and a second polypeptide chain comprises the hu6G4.2.5HV/vH1-3A and the two polypeptide chains are covalently
20 linked by one or more interchain disulfide bonds. In a preferred embodiment, the invention provides an antibody fragment comprising a plurality of polypeptide chains, wherein one polypeptide chain comprises the hu6G4.2.5LV/L1N35X₃₅ and a second polypeptide chain comprises the amino acid sequence of 6G4.2.5HV11 and the two polypeptide chains are covalently linked by one or more interchain disulfide bonds.

25 The invention further encompasses an antibody fragment comprising a plurality of polypeptide chains, wherein one polypeptide chain comprises the hu6G4.2.5LV/L1N35A and a second polypeptide chain comprises the hu6G4.2.5HV or hu6G4.2.5HV/vH1-3Z and the two polypeptide chains are covalently linked by one or more interchain disulfide bonds.

The invention also encompasses an antibody fragment comprising a plurality of polypeptide chains,
30 wherein one polypeptide chain comprises the hu6G4.2.5LV/L1N35E and a second polypeptide chain comprises the hu6G4.2.5HV and the two polypeptide chains are covalently linked by one or more interchain disulfide bonds.

In still another embodiment, the invention provides an antibody fragment comprising a plurality of polypeptide chains, wherein one polypeptide chain comprises the hu6G4.2.5LV/L1N35A and a second
35 polypeptide chain comprises the hu6G4.2.5HV/vH1-3A and the two polypeptide chains are covalently linked by one or more interchain disulfide bonds. In a preferred embodiment, the invention provides an

antibody fragment comprising a plurality of polypeptide chains, wherein one polypeptide chain comprises the hu6G4.2.5LV/L1N35A and a second polypeptide chain comprises the amino acid sequence of 6G4.2.5HV11 and the two polypeptide chains are covalently linked by one or more interchain disulfide bonds. In another preferred embodiment, the invention provides an antibody fragment comprising a plurality of polypeptide chains, wherein one polypeptide chain comprises the hu6G4.2.5LV/L1N35E and a second polypeptide chain comprises the amino acid sequence of 6G4.2.5HV11 and the two polypeptide chains are covalently linked by one or more interchain disulfide bonds.

In a preferred embodiment, any of the foregoing two-chain antibody fragments are selected from the group consisting of Fab, Fab', Fab'-SH, Fv, and F(ab')₂. In another preferred embodiment, the antibody fragment is selected from the group consisting of Fab, Fab', Fab'-SH, Fv, and F(ab')₂, wherein the antibody fragment comprises one polypeptide chain comprising the hu6G4.2.5LV/L1N35X₃₅ and a second polypeptide chain comprising the hu6G4.2.5HV. In yet another preferred embodiment, the antibody fragment is selected from the group consisting of Fab, Fab', Fab'-SH, Fv, and F(ab')₂, wherein the antibody fragment comprises one polypeptide chain comprising the hu6G4.2.5LV/L1N35A and a second polypeptide chain comprising the hu6G4.2.5HV. In a further preferred embodiment, the antibody fragment is selected from the group consisting of Fab, Fab', Fab'-SH, Fv, and F(ab')₂, wherein the antibody fragment comprises one polypeptide chain comprising the hu6G4.2.5LV/L1N35E and a second polypeptide chain comprising the hu6G4.2.5HV. In still another preferred embodiment, the antibody fragment is a F(ab')₂ that comprises one polypeptide chain comprising the hu6G4.2.5LV/L1N35A and a second polypeptide chain comprising the amino acid sequence of 6G4.2.5HV11. In an additional preferred embodiment, the antibody fragment is a F(ab')₂ that comprises one polypeptide chain comprising the hu6G4.2.5LV/L1N35E and a second polypeptide chain comprising the amino acid sequence of 6G4.2.5HV11.

The invention also provides an antibody or antibody fragment comprising a light chain variable domain containing the hu6G4.2.5LV/vL1-3X and optionally further comprising a heavy chain variable domain containing the hu6G4.2.5HV or hu6G4.2.5HV/vH1-3Z, wherein the light chain variable domain, and optionally the heavy chain variable domain, is (are) fused to an additional moiety, such as an immunoglobulin constant domain. Constant domain sequence can be added to the heavy chain and/or light chain sequence(s) to form species with full or partial length heavy and/or light chain(s). It will be appreciated that constant regions of any isotype can be used for this purpose, including IgG, IgM, IgA, IgD, and IgE constant regions, and that such constant regions can be obtained from any human or animal species. Preferably, the constant domain sequence is human in origin. Suitable human constant domain sequences can be obtained from Kabat *et al.*

The invention additionally provides an antibody or antibody fragment comprising a light chain variable domain containing the hu6G4.2.5LV/vL1-3X and optionally further comprising a heavy chain variable domain containing the hu6G4.2.5HV/vH1-3A, wherein the light chain variable domain, and

optionally the heavy chain variable domain, is (are) fused to an additional moiety, such as a immunoglobulin constant domain. Constant domain sequence can be added to the heavy chain and/or light chain sequence(s) to form species with full or partial length heavy and/or light chain(s). It will be appreciated that constant regions of any isotype can be used for this purpose, including IgG, IgM, IgA, IgD, and IgE constant regions, and that such constant regions can be obtained from any human or animal species. Preferably, the constant domain sequence is human in origin. Suitable human constant domain sequences can be obtained from Kabat *et al.*

The invention further provides an antibody or antibody fragment comprising a light chain variable domain containing the hu6G4.2.5LV/L1N35X₃₅ and optionally further comprising a heavy chain variable domain containing the hu6G4.2.5HV or hu6G4.2.5HV/vH1-3Z, wherein the light chain variable domain, and optionally the heavy chain variable domain, is (are) fused to an additional moiety, such as a immunoglobulin constant domain. Constant domain sequence can be added to the heavy chain and/or light chain sequence(s) to form species with full or partial length heavy and/or light chain(s). It will be appreciated that constant regions of any isotype can be used for this purpose, including IgG, IgM, IgA, IgD, and IgE constant regions, and that such constant regions can be obtained from any human or animal species. Preferably, the constant domain sequence is human in origin. Suitable human constant domain sequences can be obtained from Kabat *et al.*

The invention additionally provides an antibody or antibody fragment comprising a light chain variable domain containing the hu6G4.2.5LV/L1N35X₃₅ and optionally further comprising a heavy chain variable domain containing the hu6G4.2.5HV/vH1-3A, wherein the light chain variable domain, and optionally the heavy chain variable domain, is (are) fused to an additional moiety, such as a immunoglobulin constant domain. Constant domain sequence can be added to the heavy chain and/or light chain sequence(s) to form species with full or partial length heavy and/or light chain(s). It will be appreciated that constant regions of any isotype can be used for this purpose, including IgG, IgM, IgA, IgD, and IgE constant regions, and that such constant regions can be obtained from any human or animal species. Preferably, the constant domain sequence is human in origin. Suitable human constant domain sequences can be obtained from Kabat *et al.*

The invention also encompasses an antibody or antibody fragment comprising a light chain variable domain containing the hu6G4.2.5LV/L1N35A and optionally further comprising a heavy chain variable domain containing the hu6G4.2.5HV or hu6G4.2.5HV/vH1-3Z, wherein the light chain variable domain, and optionally the heavy chain variable domain, is (are) fused to an additional moiety, such as a immunoglobulin constant domain. Constant domain sequence can be added to the heavy chain and/or light chain sequence(s) to form species with full or partial length heavy and/or light chain(s). It will be appreciated that constant regions of any isotype can be used for this purpose, including IgG, IgM, IgA, IgD, and IgE constant regions, and that such constant regions can be obtained from any human or animal species. Preferably, the constant domain sequence is human in origin. Suitable human constant domain sequences can be obtained from Kabat *et al.*

The invention additionally provides an antibody or antibody fragment comprising a light chain variable domain containing the hu6G4.2.5LV/L1N35A and optionally further comprising a heavy chain variable domain containing the hu6G4.2.5HV/vH1-3A, wherein the light chain variable domain, and optionally the heavy chain variable domain, is (are) fused to an additional moiety, such as a immunoglobulin constant domain. Constant domain sequence can be added to the heavy chain and/or light chain sequence(s) to form species with full or partial length heavy and/or light chain(s). It will be appreciated that constant regions of any isotype can be used for this purpose, including IgG, IgM, IgA, IgD, and IgE constant regions, and that such constant regions can be obtained from any human or animal species. Preferably, the constant domain sequence is human in origin. Suitable human constant domain sequences can be obtained from Kabat *et al.*

The invention additionally encompasses an antibody or antibody fragment comprising a light chain variable domain containing the hu6G4.2.5LV/L1N35A and optionally further comprising a heavy chain containing the amino acid sequence of 6G4.2.5HV11, wherein the light chain variable domain, and optionally the heavy chain, is (are) fused to an additional moiety, such as immunoglobulin constant domain sequences. Constant domain sequence can be added to the heavy chain and/or light chain sequence(s) to form species with full or partial length heavy and/or light chain(s). It will be appreciated that constant regions of any isotype can be used for this purpose, including IgG, IgM, IgA, IgD, and IgE constant regions, and that such constant regions can be obtained from any human or animal species. Preferably, the constant domain sequence is human in origin. Suitable human constant domain sequences can be obtained from Kabat *et al.*

The invention further encompasses an antibody or antibody fragment comprising a light chain variable domain containing the hu6G4.2.5LV/L1N35E and optionally further comprising a heavy chain containing the amino acid sequence of 6G4.2.5HV11, wherein the light chain variable domain, and optionally the heavy chain, is (are) fused to an additional moiety, such as immunoglobulin constant domain sequences. Constant domain sequence can be added to the heavy chain and/or light chain sequence(s) to form species with full or partial length heavy and/or light chain(s). It will be appreciated that constant regions of any isotype can be used for this purpose, including IgG, IgM, IgA, IgD, and IgE constant regions, and that such constant regions can be obtained from any human or animal species. Preferably, the constant domain sequence is human in origin. Suitable human constant domain sequences can be obtained from Kabat *et al.*

In a preferred embodiment, the antibody or antibody fragment comprises a light chain variable domain containing the hu6G4.2.5LV/vL1-3X, and further comprises the hu6G4.2.5HV or hu6G4.2.5HV/vH1-3Z in a heavy chain that is fused to or contains a leucine zipper sequence. The leucine zipper can increase the affinity or production efficiency of the antibody or antibody fragment of interest. Suitable leucine zipper sequences include the jun and fos leucine zippers taught by Kostelney *et al.*, J. Immunol., 148: 1547-1553 (1992) and the GCN4 leucine zipper described in the Examples below.

In particular, the invention provides an antibody or antibody fragment comprising a light chain

comprising the amino acid sequence of amino acids 1-219 of the variant humanized anti-IL-8 6G4.2.5v11 light chain polypeptide amino acid sequence of Fig. 31B (SEQ ID NO: 65) with the proviso that any amino acid other than Asn (denoted as "X₃₅") is substituted for Asn at amino acid position 35 (herein referred to as "6G4.2.5LV11N35X₃₅").

5 In another embodiment, the invention provides an antibody or antibody fragment comprising a light chain comprising the amino acid sequence of amino acids 1-219 of the variant humanized anti-IL-8 6G4.2.5v11 light chain polypeptide amino acid sequence of Fig. 31B (SEQ ID NO: 65) with the proviso that any amino acid other than Ser (denoted as "X₂₆") is substituted for Ser at amino acid position 26 (herein referred to as "6G4.2.5LV11S26X₂₆").

10 In yet another embodiment, the invention provides an antibody or antibody fragment comprising a light chain comprising the amino acid sequence of amino acids 1-219 of the variant humanized anti-IL-8 6G4.2.5v11 light chain polypeptide amino acid sequence of Fig. 31B (SEQ ID NO: 65) with the proviso that any amino acid other than His (denoted as "X₉₈") is substituted for His at amino acid position 98 (herein referred to as "6G4.2.5LV11H98X₉₈").

15 In still another embodiment, the invention provides an antibody or antibody fragment comprising a light chain comprising the amino acid sequence of amino acids 1-219 of the variant humanized anti-IL-8 6G4.2.5v11 light chain polypeptide amino acid sequence of Fig. 31B (SEQ ID NO: 65) with the proviso that any amino acid other than Ser (denoted as "X₂₆") is substituted for Ser at amino acid position 26 and any amino acid other than Asn (denoted as "X₃₅") is substituted for Asn at amino acid position 35 (herein referred to as "6G4.2.5LV11S26X₂₆/N35X₃₅").

20 In a further embodiment, the invention provides an antibody or antibody fragment comprising a light chain comprising the amino acid sequence of amino acids 1-219 of the variant humanized anti-IL-8 6G4.2.5v11 light chain polypeptide amino acid sequence of Fig. 31B (SEQ ID NO: 65) with the proviso that any amino acid other than Asn (denoted as "X₃₅") is substituted for Asn at amino acid position 35 and any amino acid other than His (denoted as "X₉₈") is substituted for His at amino acid position 98 (herein referred to as "6G4.2.5LV11N35X₃₅/H98X₉₈").

25 In an additional embodiment, the invention provides an antibody or antibody fragment comprising a light chain comprising the amino acid sequence of amino acids 1-219 of the variant humanized anti-IL-8 6G4.2.5v11 light chain polypeptide amino acid sequence of Fig. 31B (SEQ ID NO: 65) with the proviso that any amino acid other than Ser (denoted as "X₂₆") is substituted for Ser at amino acid position 26 and any amino acid other than His (denoted as "X₉₈") is substituted for His at amino acid position 98 (herein referred to as "6G4.2.5LV11S26X₂₆/H98X₉₈").

The invention also encompasses an antibody or antibody fragment comprising a light chain comprising the amino acid sequence of amino acids 1-219 of the variant humanized anti-IL-8 6G4.2.5v11 light chain polypeptide amino acid sequence of Fig. 31B (SEQ ID NO: 65) with the proviso that any amino acid other than Ser (denoted as "X₂₆") is substituted for Ser at amino acid position 26, any amino acid other than Asn (denoted as "X₃₅") is substituted for Asn at amino acid position 35 and any amino acid other than His (denoted as "X₉₈") is substituted for His at amino acid position 98 (herein referred to as "6G4.2.5LV11S26X₂₆/N35X₃₅/H98X₉₈").

Additionally, the invention provides an antibody or antibody fragment comprising a light chain comprising the amino acid sequence of amino acids 1-219 of the variant humanized anti-IL-8 6G4.2.5v11 light chain polypeptide amino acid sequence (SEQ ID NO: 71) of Fig. 36 (herein referred to as "6G4.2.5LV11N35A").

Further provided herein is an antibody or antibody fragment comprising a light chain comprising the amino acid sequence of amino acids 1-219 of the variant humanized anti-IL-8 6G4.2.5v11 light chain polypeptide amino acid sequence (SEQ ID NO: 71) of Fig. 45 (herein referred to as "6G4.2.5LV11N35E").

In another embodiment, the invention provides an antibody or antibody fragment comprising a light chain comprising the amino acid sequence of amino acids 1-219 of the variant humanized anti-IL-8 6G4.2.5v11 light chain polypeptide amino acid sequence of Fig. 31B (SEQ ID NO: 65) with the proviso that Ala is substituted for Ser at amino acid position 26 (herein referred to as "6G4.2.5LV11S26A").

In yet another embodiment, the invention provides an antibody or antibody fragment comprising a light chain comprising the amino acid sequence of amino acids 1-219 of the variant humanized anti-IL-8 6G4.2.5v11 light chain polypeptide amino acid sequence of Fig. 31B (SEQ ID NO: 65) with the proviso that Ala is substituted for His at amino acid position 98 (herein referred to as "6G4.2.5LV11H98A").

In still another embodiment, the invention provides an antibody or antibody fragment comprising a light chain comprising the amino acid sequence of amino acids 1-219 of the variant humanized anti-IL-8 6G4.2.5v11 light chain polypeptide amino acid sequence of Fig. 31B (SEQ ID NO: 65) with the proviso that Ala is substituted for Ser at amino acid position 26 and Ala is substituted for Asn at amino acid position 35 (herein referred to as "6G4.2.5LV11S26A/N35A").

In a further embodiment, the invention provides an antibody or antibody fragment comprising a light chain comprising the amino acid sequence of amino acids 1-219 of the variant humanized anti-IL-8 6G4.2.5v11 light chain polypeptide amino acid sequence of Fig. 31B (SEQ ID NO: 65) with the proviso that Ala is substituted for Ser at amino acid position 26 and Ala is substituted for His at amino acid position 98 (herein referred to as "6G4.2.5LV11S26A/H98A").

The invention also encompasses an antibody or antibody fragment comprising a light chain comprising the amino acid sequence of amino acids 1-219 of the variant humanized anti-IL-8 6G4.2.5v11 light chain polypeptide amino acid sequence of Fig. 31B (SEQ ID NO: 65) with the proviso that Ala is substituted for Asn at amino acid position 35 and Ala is substituted for His at amino acid position 98 (herein

referred to as "6G4.2.5LV11N35A/H98A").

The invention further encompasses an antibody or antibody fragment comprising a light chain comprising the amino acid sequence of amino acids 1-219 of the variant humanized anti-IL-8 6G4.2.5v11 light chain polypeptide amino acid sequence of Fig. 31B (SEQ ID NO: 65) with the proviso that Ala is substituted for Ser at amino acid position 26, Ala is substituted for Asn at amino acid position 35, and Ala is substituted for His at amino acid position 98 (herein referred to as "6G4.2.5LV11S26A/N35A/H98A").

The invention provides a single chain antibody fragment comprising a variant light chain selected from the group consisting of 6G4.2.5LV11N35X₃₅, 6G4.2.5LV11S26X₂₆, 6G4.2.5LV11H98X₉₈, 6G4.2.5LV11S26X₂₆/ N35X₃₅, 6G4.2.5LV11N35X₃₅/ H98X₉₈, 6G4.2.5LV11S26X₂₆/H98X₉₈, and 6G4.2.5LV11S26X₂₆/ N35X₃₅/H98X₉₈, with or without any additional amino acid sequence. It will be understood that the group consisting of 6G4.2.5LV11N35X₃₅, 6G4.2.5LV11S26X₂₆, 6G4.2.5LV11H98X₉₈, 6G4.2.5LV11S26X₂₆/ N35X₃₅, 6G4.2.5LV11N35X₃₅/ H98X₉₈, 6G4.2.5LV11S26X₂₆/H98X₉₈, and 6G4.2.5LV11S26X₂₆/ N35X₃₅/H98X₉₈, is collectively referred to herein as the "group of 6G4.2.5LV11X variants", and that individual members of this group are generically referred to herein as a "6G4.2.5LV11X variant." In one embodiment, the invention provides a single chain antibody fragment comprising a 6G4.2.5LV11X variant without any associated heavy chain amino acid sequence, i.e. a single chain species that makes up one half of a Fab fragment. In a preferred embodiment, the invention provides a 6G4.2.5LV11N35X₃₅ variant without any associated heavy chain amino acid sequence.

The invention encompasses a single chain antibody fragment comprising a variant light chain selected from the group consisting of 6G4.2.5LV11N35A, 6G4.2.5LV11S26A, 6G4.2.5LV11H98A, 6G4.2.5LV11S26A/ N35A, 6G4.2.5LV11N35A/ H98A, 6G4.2.5LV11S26A/H98A, and 6G4.2.5LV11S26A/ N35A/H98A, with or without any additional amino acid sequence. It will be understood that the group consisting of 6G4.2.5LV11N35A, 6G4.2.5LV11S26A, 6G4.2.5LV11H98A, 6G4.2.5LV11S26A/ N35A, 6G4.2.5LV11N35A/ H98A, 6G4.2.5LV11S26A/H98A, and 6G4.2.5LV11S26A/ N35A/H98A is collectively referred to herein as the "group of 6G4.2.5LV11A variants", and that individual members of this group are generically referred to herein as a "6G4.2.5LV11A variant." In one embodiment, the invention provides a single chain antibody fragment comprising a 6G4.2.5LV11A variant without any associated heavy chain amino acid sequence, i.e. a single chain species that makes up one half of a Fab fragment. In a preferred embodiment, the invention provides the 6G4.2.5LV11N35A without any associated heavy chain amino acid sequence.

Further provided herein are an antibody or antibody fragment comprising a light chain comprising a 6G4.2.5LV11X variant, and further comprising a heavy chain comprising the 6G4.2.5HV11. In a preferred embodiment, the invention provides an antibody or antibody fragment comprising a 6G4.2.5LV11N35X₃₅ variant and further comprising the 6G4.2.5HV11. In a preferred embodiment, the

invention provides an antibody or antibody fragment comprising the 6G4.2.5LV11N35A and further comprising the 6G4.2.5HV11. In another preferred embodiment, the invention provides an antibody or antibody fragment comprising the 6G4.2.5LV11N35E and further comprising the 6G4.2.5HV11.

In one embodiment, the invention provides a single chain antibody fragment wherein a
5 6G4.2.5LV11X variant and the 6G4.2.5HV11 are contained in a single chain polypeptide species. In a preferred embodiment, the single chain antibody fragment comprises a 6G4.2.5LV11X variant joined to the 6G4.2.5HV11 by means of a flexible peptide linker sequence, wherein the heavy chain and light chain domains can associate in a "dimeric" structure analogous to that formed in a two-chain Fab species. In another embodiment, the single chain antibody fragment is a species comprising a 6G4.2.5LV11X variant
10 joined to the 6G4.2.5HV11 by a linker that is too short to permit intramolecular pairing of complementary domains, i.e. a single chain polypeptide monomer that forms a diabody upon dimerization with another monomer.

In still another embodiment, the invention provides a single chain antibody fragment wherein a 6G4.2.5LV11N35X₃₅ variant and the 6G4.2.5HV11 are contained in a single chain polypeptide species. In
15 a preferred embodiment, the single chain antibody fragment comprises a 6G4.2.5LV11N35X₃₅ variant joined to the 6G4.2.5HV11 by means of a flexible peptide linker sequence, wherein the heavy chain and light chain domains can associate in a "dimeric" structure analogous to that formed in a two-chain Fab species. In another embodiment, the single chain antibody fragment is a species comprising a 6G4.2.5LV11N35X₃₅ variant joined to the 6G4.2.5HV11 by a linker that is too short to permit
20 intramolecular pairing of complementary domains, i.e. a single chain polypeptide monomer that forms a diabody upon dimerization with another monomer.

In a further embodiment, the invention provides a single chain antibody fragment wherein the 6G4.2.5LV11N35A and the 6G4.2.5HV11 are contained in a single chain polypeptide species. In a preferred embodiment, the single chain antibody fragment comprises the 6G4.2.5LV11N35A joined to the
25 6G4.2.5HV11 by means of a flexible peptide linker sequence, wherein the heavy chain and light chain domains can associate in a "dimeric" structure analogous to that formed in a two-chain Fab species. In another embodiment, the single chain antibody fragment is a species comprising the 6G4.2.5LV11N35A joined to the 6G4.2.5HV11 by a linker that is too short to permit intramolecular pairing of complementary domains, i.e. a single chain polypeptide monomer that forms a diabody upon dimerization with another
30 monomer.

In an additional embodiment, the invention provides a single chain antibody fragment wherein the 6G4.2.5LV11N35E and the 6G4.2.5HV11 are contained in a single chain polypeptide species. In a preferred embodiment, the single chain antibody fragment comprises the 6G4.2.5LV11N35E joined to the 6G4.2.5HV11 by means of a flexible peptide linker sequence, wherein the heavy chain and light chain
35 domains can associate in a "dimeric" structure analogous to that formed in a two-chain Fab species. In another embodiment, the single chain antibody fragment is a species comprising the 6G4.2.5LV11N35E

joined to the 6G4.2.5HV11 by a linker that is too short to permit intramolecular pairing of complementary domains, i.e. a single chain polypeptide monomer that forms a diabody upon dimerization with another monomer.

In yet another embodiment, the invention provides an antibody fragment comprising a plurality of polypeptide chains, wherein one polypeptide chain comprises a 6G4.2.5LV11X variant and a second polypeptide chain comprises the 6G4.2.5HV11 and the two polypeptide chains are covalently linked by one or more interchain disulfide bonds. In still another embodiment, the invention provides an antibody fragment comprising a plurality of polypeptide chains, wherein one polypeptide chain comprises a 6G4.2.5LV11N35X₃₅ variant and a second polypeptide chain comprises the 6G4.2.5HV11 and the two polypeptide chains are covalently linked by one or more interchain disulfide bonds. In a preferred embodiment, any of the foregoing two-chain antibody fragments is selected from the group consisting of Fab, Fab', Fab'-SH, and F(ab')₂. In still another preferred embodiment, the two-chain antibody fragment is a F(ab')₂ wherein one polypeptide chain comprises the 6G4.2.5LV11N35A and the second polypeptide chain comprises the 6G4.2.5HV11. In a further preferred embodiment, the antibody fragment is a Fab, Fab', Fab'-SH, or F(ab')₂ wherein one polypeptide chain comprises the 6G4.2.5LV11N35E and the second polypeptide chain comprises the 6G4.2.5HV11. A particularly preferred embodiment, the antibody fragment is the 6G4V11N35A F(ab')₂ GCN4 leucine zipper species described in the Examples below. In another particularly preferred embodiment, the antibody fragment is the 6G4V11N35E F(ab')₂ GCN4 leucine zipper species described in the Examples below. In yet another particularly preferred embodiment, the antibody fragment is the 6G4V11N35E Fab described in the Examples below.

The invention also provides an antibody or antibody fragment comprising a light chain containing a 6G4.2.5LV11X variant and optionally further comprising a heavy chain containing the 6G4.2.5HV11, wherein the light chain, and optionally the heavy chain, is (are) fused to an additional moiety, such as additional immunoglobulin constant domain sequence. Constant domain sequence can be added to the heavy chain and/or light chain sequence(s) to form species with full or partial length heavy and/or light chain(s). It will be appreciated that constant regions of any isotype can be used for this purpose, including IgG, IgM, IgA, IgD, and IgE constant regions, and that such constant regions can be obtained from any human or animal species. Preferably, the constant domain sequence is human in origin. Suitable human constant domain sequences can be obtained from Kabat *et al.*

The invention additionally provides an antibody or antibody fragment comprising a light chain containing a 6G4.2.5LV11N35X₃₅ variant and optionally further comprising a heavy chain containing the 6G4.2.5HV11, wherein the light chain, and optionally the heavy chain, is (are) fused to an additional moiety, such as additional immunoglobulin constant domain sequence. Constant domain sequence can be added to the heavy chain and/or light chain sequence(s) to form species with full or partial length heavy and/or light chain(s). It will be appreciated that constant regions of any isotype can be used for this purpose,

including IgG, IgM, IgA, IgD, and IgE constant regions, and that such constant regions can be obtained from any human or animal species. Preferably, the constant domain sequence is human in origin. Suitable human constant domain sequences can be obtained from Kabat *et al.*

5 The invention further provides an antibody or antibody fragment comprising a light chain containing the 6G4.2.5LV11N35A and optionally further comprising a heavy chain containing the 6G4.2.5HV11, wherein the light chain, and optionally the heavy chain, is (are) fused to an additional moiety, such as additional immunoglobulin constant domain sequence. Constant domain sequence can be added to the heavy chain and/or light chain sequence(s) to form species with full or partial length heavy and/or light chain(s). It will be appreciated that constant regions of any isotype can be used for this purpose, 10 including IgG, IgM, IgA, IgD, and IgE constant regions, and that such constant regions can be obtained from any human or animal species. Preferably, the constant domain sequence is human in origin. Suitable human constant domain sequences can be obtained from Kabat *et al.*

The invention further provides an antibody or antibody fragment comprising a light chain containing the 6G4.2.5LV11N35E and optionally further comprising a heavy chain containing the 15 6G4.2.5HV11, wherein the light chain, and optionally the heavy chain, is (are) fused to an additional moiety, such as additional immunoglobulin constant domain sequence. Constant domain sequence can be added to the heavy chain and/or light chain sequence(s) to form species with full or partial length heavy and/or light chain(s). It will be appreciated that constant regions of any isotype can be used for this purpose, including IgG, IgM, IgA, IgD, and IgE constant regions, and that such constant regions can be obtained 20 from any human or animal species. Preferably, the constant domain sequence is human in origin. Suitable human constant domain sequences can be obtained from Kabat *et al.*

In a preferred embodiment, the antibody or antibody fragment comprises a light chain containing a 6G4.2.5LV11X variant, and further comprises the 6G4.2.5HV11 in a heavy chain that is fused to or contains a leucine zipper sequence. The leucine zipper can increase the affinity or production efficiency of the 25 antibody or antibody fragment of interest. Suitable leucine zipper sequences include the jun and fos leucine zippers taught by Kostelney *et al.*, *J. Immunol.*, 148: 1547-1553 (1992) and the GCN4 leucine zipper described in the Examples below. In another preferred embodiment, the antibody or antibody fragment comprises a light chain containing the 6G4.2.5LV11N35A, and further comprises a heavy chain containing the 6G4.2.5HV11 fused to the GCN4 leucine zipper. In yet another preferred embodiment, the antibody or 30 antibody fragment comprises a light chain containing the 6G4.2.5LV11N35E, and further comprises a heavy chain containing the 6G4.2.5HV11 fused to the GCN4 leucine zipper.

B. 6G4.2.5HV VARIANTS

The invention provides humanized antibodies and antibody fragments comprising the CDRs of a 6G4.2.5HV CDR variant. The use of a 6G4.2.5HV CDRs variant in the humanized antibodies 35 and antibody fragments of the invention confer the advantages of higher affinity for human IL-8 and/or improved recombinant manufacturing economy.

A heavy chain variable domain comprising the CDRs of a 6G4.2.5HV CDRs variant can be

humanized in conjunction with a light chain comprising the CDRs of 6G4.2.5LV or the CDRs of a 6G4.2.5LV CDRs variant, essentially as described in Section (II)(2)(A) above. In one embodiment, the invention provides a humanized antibody or antibody fragment comprising a 6G4.2.5HV CDRs variant selected from the group consisting of 6G4.2.5HV/H1S31Z₃₁, 6G4.2.5HV/H2S54Z₅₄, and

5 6G4.2.5HV/H1S31Z₃₁/H2S54Z₅₄. In addition, the invention provides a humanized antibody or antibody fragment comprising a 6G4.2.5HV CDRs variant selected from the group consisting of 6G4.2.5HV/H1S31A, 6G4.2.5HV/H2S54A, and 6G4.2.5HV/H1S31A/H2S54A. In particular, the 6G4.2.5HV CDRs variants can be used to construct a humanized antibody or antibody comprising the hu6G4.2.5HV/vH1-3Z as described in Section (II)(2)(A) above.

10 The invention additionally provides a humanized antibody or antibody fragment that comprises a heavy chain variable domain comprising the hu6G4.2.5HV/vH1-3Z, and further comprises a light chain variable domain comprising the hu6G4.2.5LV or hu6G4.2.5LV/vL1-3X.

The invention further encompasses a single chain humanized antibody fragment comprising the hu6G4.2.5HV/vH1-3Z, with or without any additional amino acid sequence. In one embodiment, the
15 invention provides a single chain antibody fragment comprising the hu6G4.2.5HV/vH1-3Z without any associated heavy chain variable domain amino acid sequence, i.e. a single chain species that makes up one half of an Fv fragment.

In one embodiment, the invention provides a single chain humanized antibody fragment wherein the hu6G4.2.5HV/vH1-3Z and the hu6G4.2.5LV or hu6G4.2.5LV/vL1-3X are contained in a single chain
20 polypeptide species. In a preferred embodiment, the single chain antibody fragment is a scFv species comprising the hu6G4.2.5HV/vH1-3Z joined to the hu6G4.2.5LV or hu6G4.2.5LV/vL1-3X by means of a flexible peptide linker sequence, wherein the heavy chain and light chain variable domains can associate in a "dimeric" structure analogous to that formed in a two-chain Fv species. In another embodiment, the single chain antibody fragment is a species comprising the hu6G4.2.5HV/vH1-3Z joined to the hu6G4.2.5LV or
25 hu6G4.2.5LV/vL1-3X by a linker that is too short to permit intramolecular pairing of the two variable domains, i.e. a single chain polypeptide monomer that forms a diabody upon dimerization with another monomer.

In yet another embodiment, the invention provides a humanized antibody fragment comprising a plurality of polypeptide chains, wherein one polypeptide chain comprises the hu6G4.2.5HV/vH1-3Z and a
30 second polypeptide chain comprises the hu6G4.2.5LV or hu6G4.2.5LV/vL1-3X and the two polypeptide chains are covalently linked by one or more interchain disulfide bonds. In a preferred embodiment, the foregoing two-chain antibody fragment is selected from the group consisting of Fab, Fab', Fab'-SH, Fv, and F(ab')₂.

The invention also provides a humanized antibody or antibody fragment comprising a heavy chain
35 variable domain containing the hu6G4.2.5HV/vH1-3Z and optionally further comprising a light chain variable domain containing the hu6G4.2.5LV or hu6G4.2.5LV/vL1-3X, wherein the heavy chain variable

domain, and optionally the light chain variable domain, is (are) fused to an additional moiety, such as an immunoglobulin constant domain. Constant domain sequence can be added to the heavy chain and/or light chain sequence(s) to form species with full or partial length heavy and/or light chain(s). It will be appreciated that constant regions of any isotype can be used for this purpose, including IgG, IgM, IgA, IgD, and IgE constant regions, and that such constant regions can be obtained from any human or animal species. Preferably, the constant domain sequence is human in origin. Suitable human constant domain sequences can be obtained from Kabat *et al.*

In a preferred embodiment, the humanized antibody or antibody fragment comprises the hu6G4.2.5HV/vH1-3Z in a heavy chain that is fused to or contains a leucine zipper sequence. The leucine zipper can increase the affinity or production efficiency of the antibody or antibody fragment of interest. Suitable leucine zipper sequences include the jun and fos leucine zippers taught by Kostelney *et al.*, J. Immunol., 148: 1547-1553 (1992) and the GCN4 leucine zipper described in the Examples below.

In addition, the invention provides a humanized antibody or antibody fragment comprising a heavy chain comprising the amino acid sequence of amino acids 1-230 of the 6G4.2.5HV11 polypeptide amino acid sequence of Figs. 37A-37B (SEQ ID NO: 75) with the proviso that Ala is substituted for Ser at amino acid position 31 (hereinafter referred to as "6G4.2.5HV11S31A").

In another embodiment, the invention provides a humanized antibody or antibody fragment comprising a heavy chain comprising the amino acid sequence of amino acids 1-230 of the 6G4.2.5HV11 polypeptide amino acid sequence of Figs. 37A-37B (SEQ ID NO: 75) with the proviso that Ala is substituted for Ser at amino acid position 54 (hereinafter referred to as "6G4.2.5HV11S54A").

In yet another embodiment, the invention provides a humanized antibody or antibody fragment comprising a heavy chain comprising the amino acid sequence of amino acids 1-230 of the 6G4.2.5HV11 polypeptide amino acid sequence of Figs. 37A-37B (SEQ ID NO: 75) with the proviso that Ala is substituted for Ser at amino acid position 31 and Ala is substituted for Ser at amino acid position 54 (hereinafter referred to as "6G4.2.5HV11S31A/S54A").

Further provided herein is a humanized antibody or antibody fragment that comprises any of the light and heavy chain combinations listed in Tables 1 and 2 below.

Table 1

	Heavy Chain	Light Chain
30	6G4.2.5HV11S31A	6G4.2.5LV11
	6G4.2.5HV11S31A	6G4.2.5LV11N35A
	6G4.2.5HV11S31A	6G4.2.5LV11S26A
	6G4.2.5HV11S31A	6G4.2.5LV11H98A
35	6G4.2.5HV11S31A	6G4.2.5LV11S26A/N35A
	6G4.2.5HV11S31A	6G4.2.5LV11S26A/H98A
	6G4.2.5HV11S31A	6G4.2.5LV11N35A/H98A
	6G4.2.5HV11S31A	6G4.2.5LV11S26A/N35A/H98A
	6G4.2.5HV11S54A	6G4.2.5LV11
40	6G4.2.5HV11S54A	6G4.2.5LV11N35A
	6G4.2.5HV11S54A	6G4.2.5LV11S26A
	6G4.2.5HV11S54A	6G4.2.5LV11H98A

Table 2

	Heavy Chain	Light Chain
	6G4.2.5HV11S54A	6G4.2.5LV11S26A/N35A
5	6G4.2.5HV11S54A	6G4.2.5LV11S26A/H98A
	6G4.2.5HV11S54A	6G4.2.5LV11N35A/H98A
	6G4.2.5HV11S54A	6G4.2.5LV11S26A/N35A/H98A
	6G4.2.5HV11S31A/S54A	6G4.2.5LV11
	6G4.2.5HV11S31A/S54A	6G4.2.5LV11N35A
10	6G4.2.5HV11S31A/S54A	6G4.2.5LV11S26A
	6G4.2.5HV11S31A/S54A	6G4.2.5LV11H98A
	6G4.2.5HV11S31A/S54A	6G4.2.5LV11S26A/N35A
	6G4.2.5HV11S31A/S54A	6G4.2.5LV11S26A/H98A
	6G4.2.5HV11S31A/S54A	6G4.2.5LV11N35A/H98A
15	6G4.2.5HV11S31A/S54A	6G4.2.5LV11S26A/N35A/H98A
	6G4.2.5HV11S31A	6G4.2.5LV11
	6G4.2.5HV11S31A	6G4.2.5LV11N35X ₃₅
	6G4.2.5HV11S31A	6G4.2.5LV11S26X ₂₆
	6G4.2.5HV11S31A	6G4.2.5LV11H98X ₉₈
20	6G4.2.5HV11S31A	6G4.2.5LV11S26X ₂₆ /N35X ₃₅
	6G4.2.5HV11S31A	6G4.2.5LV11S26X ₂₆ /H98X ₉₈
	6G4.2.5HV11S31A	6G4.2.5LV11N35X ₃₅ /H98X ₉₈
	6G4.2.5HV11S31A	6G4.2.5LV11S26X ₂₆ /N35X ₃₅ /H98X ₉₈
	6G4.2.5HV11S54A	6G4.2.5LV11
25	6G4.2.5HV11S54A	6G4.2.5LV11N35X ₃₅
	6G4.2.5HV11S54A	6G4.2.5LV11S26X ₂₆
	6G4.2.5HV11S54A	6G4.2.5LV11H98X ₉₈
	6G4.2.5HV11S54A	6G4.2.5LV11S26X ₂₆ /N35X ₃₅
	6G4.2.5HV11S54A	6G4.2.5LV11S26X ₂₆ /H98X ₉₈
30	6G4.2.5HV11S54A	6G4.2.5LV11N35X ₃₅ /H98X ₉₈
	6G4.2.5HV11S54A	6G4.2.5LV11S26X ₂₆ /N35X ₃₅ /H98X ₉₈
	6G4.2.5HV11S31A/S54A	6G4.2.5LV11
	6G4.2.5HV11S31A/S54A	6G4.2.5LV11N35X ₃₅
	6G4.2.5HV11S31A/S54A	6G4.2.5LV11S26X ₂₆
35	6G4.2.5HV11S31A/S54A	6G4.2.5LV11H98X ₉₈
	6G4.2.5HV11S31A/S54A	6G4.2.5LV11S26X ₂₆ /N35X ₃₅
	6G4.2.5HV11S31A/S54A	6G4.2.5LV11S26X ₂₆ /H98X ₉₈
	6G4.2.5HV11S31A/S54A	6G4.2.5LV11N35X ₃₅ /H98X ₉₈
	6G4.2.5HV11S31A/S54A	6G4.2.5LV11S26X ₂₆ /N35X ₃₅ /H98X ₉₈
40		

The invention encompasses a single chain humanized antibody fragment comprising a variant heavy chain selected from the group consisting of 6G4.2.5HV11S31A, 6G4.2.5HV11S54A, and 6G4.2.5HV11S31A/S54A, with or without any additional amino acid sequence. It will be understood that the group consisting of 6G4.2.5HV11S31A, 6G4.2.5HV11S54A, and 6G4.2.5HV11S31A/S54A is collectively referred to herein as the "group of 6G4.2.5HV11A variants", and that individual members of

this group are generically referred to herein as a "6G4.2.5HV11A variant." In one embodiment, the invention provides a single chain humanized antibody fragment comprising a 6G4.2.5HV11A variant without any associated light chain amino acid sequence, i.e. a single chain species that makes up one half of a Fab fragment.

5 Further provided herein are a humanized antibody or antibody fragment comprising a heavy chain comprising a 6G4.2.5HV11A variant, and further comprising a light chain comprising a 6G4.2.5LV11A variant or a 6G4.2.5LV11X variant. In another embodiment, the humanized antibody or antibody fragment comprises any combination of light and heavy chains listed in Tables 1 and 2 above. In one embodiment, the invention provides a humanized antibody or antibody fragment comprising a 6G4.2.5HV11A variant
10 and further comprising the 6G4.2.5LV11N35X₃₅. In a preferred embodiment, the invention provides a humanized antibody or antibody fragment comprising a 6G4.2.5HV11A variant and further comprising the 6G4.2.5LV11N35A.

In yet another embodiment, the invention provides a single chain humanized antibody fragment wherein a 6G4.2.5HV11A variant and the 6G4.2.5LV11 are contained in a single chain polypeptide species.
15 In another embodiment, the invention provides a single chain humanized antibody fragment wherein any pair of light and heavy chains listed in Tables 1 and 2 above is contained in a single chain polypeptide species. In yet another embodiment, the invention provides a single chain humanized antibody fragment wherein a 6G4.2.5HV11A variant and a 6G4.2.5LV11X variant are contained in a single chain polypeptide species. In still another embodiment, the invention provides a single chain humanized antibody fragment
20 wherein a 6G4.2.5HV11A variant and a 6G4.2.5LV11N35X₃₅ variant are contained in a single chain polypeptide species. In an additional embodiment, the invention provides a single chain humanized antibody fragment wherein a 6G4.2.5HV11A variant and the 6G4.2.5LV11N35A variant are contained in a single chain polypeptide species.

In a preferred embodiment, the single chain humanized antibody fragment comprises a
25 6G4.2.5HV11A variant joined to a 6G4.2.5LV11X variant, 6G4.2.5LV11N35X₃₅ variant, 6G4.2.5LV11N35A variant, or 6G4.2.5LV11 by means of a flexible peptide linker sequence, wherein the heavy chain and light chain domains can associate in a "dimeric" structure analogous to that formed in a two-chain Fab species. In a further embodiment, the single chain humanized antibody fragment is a species comprising a 6G4.2.5HV11A variant joined to a 6G4.2.5LV11X variant, 6G4.2.5LV11N35X₃₅ variant,
30 6G4.2.5LV11N35A variant, or 6G4.2.5LV11 by a linker that is too short to permit intramolecular pairing of complementary domains, i.e. a single chain polypeptide monomer that forms a diabody upon dimerization with another monomer.

In still another embodiment, the single chain humanized antibody fragment comprises any pair of light and heavy chains listed in Tables 1 and 2 above joined by means of a flexible peptide linker sequence,
-35 wherein the heavy chain and light chain domains can associate in a "dimeric" structure analogous to that formed in a two-chain Fab species. In an additional embodiment, the single chain humanized antibody

fragment comprises any pair of light and heavy chains listed in Tables 1 and 2 above joined by a linker that is too short to permit intramolecular pairing of complementary domains, i.e. a single chain polypeptide monomer that forms a diabody upon dimerization with another monomer.

5 In yet another embodiment, the invention provides a humanized antibody fragment comprising a plurality of polypeptide chains, wherein one polypeptide chain comprises a 6G4.2.5HV11A variant and a second polypeptide chain comprises a 6G4.2.5LV11X variant, 6G4.2.5LV11N35X₃₅ variant, 6G4.2.5LV11N35A variant, or 6G4.2.5LV11, and the two polypeptide chains are covalently linked by one or more interchain disulfide bonds. In a preferred embodiment, the foregoing two-chain antibody fragment is selected from the group consisting of Fab, Fab', Fab'-SH, and F(ab')₂.

10 In an additional embodiment, the invention provides a two-chain humanized antibody fragment comprising any pair of heavy and light chains listed in Tables 1 and 2 above, wherein each chain is contained on a separate molecule. In another embodiment, the two-chain antibody fragment comprising any pair of heavy and light chains listed in Tables 1 and 2 above is selected from the group consisting of Fab, Fab', Fab'-SH, and F(ab')₂. In a preferred embodiment, the two-chain humanized antibody fragment is a F(ab')₂ comprising any pair of heavy and light chains listed in Tables 1 and 2 above. In another preferred embodiment, the two-chain humanized antibody fragment is a F(ab')₂ wherein one polypeptide chain comprises a 6G4.2.5HV11A variant and the second polypeptide chain comprises the 6G4.2.5LV11N35A.

The invention also provides a humanized antibody or antibody fragment comprising a heavy chain containing a 6G4.2.5HV11A variant and optionally further comprising a light chain containing a 6G4.2.5LV11X variant, 6G4.2.5LV11N35X₃₅ variant, 6G4.2.5LV11N35A, or 6G4.2.5HV11, wherein the heavy chain, and optionally the light chain, is (are) fused to an additional moiety, such as additional immunoglobulin constant domain sequence. Constant domain sequence can be added to the heavy chain and/or light chain sequence(s) to form species with full or partial length heavy and/or light chain(s). It will be appreciated that constant regions of any isotype can be used for this purpose, including IgG, IgM, IgA, 20 IgD, and IgE constant regions, and that such constant regions can be obtained from any human or animal species. Preferably, the constant domain sequence is human in origin. Suitable human constant domain sequences can be obtained from Kabat *et al.* (supra).

In a preferred embodiment, the humanized antibody or antibody fragment comprises a 6G4.2.5HV11A variant in a heavy chain that is fused to or contains a leucine zipper sequence. The leucine zipper can increase the affinity or production efficiency of the antibody or antibody fragment of interest. Suitable leucine zipper sequences include the jun and fos leucine zippers taught by Kostelny *et al.*, J. Immunol., 148: 1547-1553 (1992) and the GCN4 leucine zipper described in the Examples below.

C. Bispecific Antibodies

Bispecific antibodies are monoclonal, preferably human or humanized, antibodies that 35 have binding specificities for at least two different antigens. In the present case, one of the binding

specificities is for IL-8, the other one is for any other antigen. For example, bispecific antibodies specifically binding a IL-8 and neurotrophic factor, or two different types of IL-8 polypeptides are within the scope of the present invention.

Methods for making bispecific antibodies are known in the art. Traditionally, the recombinant production of bispecific antibodies is based on the co-expression of two immunoglobulin heavy chain-light chain pairs, where the two heavy chains have different specificities (Milstein and Cuello, Nature 305:537 (1983)). Because of the random assortment of immunoglobulin heavy and light chains, these hybridomas (quadromas) produce a potential mixture of 10 different antibody molecules, of which only one has the correct bispecific structure. The purification of the correct molecule, which is usually done by affinity chromatography steps, is rather cumbersome, and the product yields are low. Similar procedures are disclosed in WO 93/08829 published 13 May 1993, and in Traunecker *et al.*, EMBO J. 10:3655 (1991).

According to a different and more preferred approach, antibody-variable domains with the desired binding specificities (antibody-antigen combining sites) are fused to immunoglobulin constant-domain sequences. The fusion preferably is with an immunoglobulin heavy-chain constant domain, comprising at least part of the hinge, CH2, and CH3 regions. It is preferred to have the first heavy-chain constant region (CH1), containing the site necessary for light-chain binding, present in at least one of the fusions. DNAs encoding the immunoglobulin heavy chain fusions and, if desired, the immunoglobulin light chain, are inserted into separate expression vectors, and are co-transfected into a suitable host organism. This provides for great flexibility in adjusting the mutual proportions of the three polypeptide fragments in embodiments when unequal ratios of the three polypeptide chains used in the construction provide the maximum yields. It is, however, possible to insert the coding sequences for two or all three polypeptide chains in one expression vector when the production of at least two polypeptide chains in equal ratios results in high yields or when the ratios are of no particular significance. In a preferred embodiment of this approach, the bispecific antibodies are composed of a hybrid immunoglobulin heavy chain with a first binding specificity in one arm, and a hybrid immunoglobulin heavy chain-light chain pair (providing a second binding specificity) in the other arm. This asymmetric structure facilitates the separation of the desired bispecific compound from unwanted immunoglobulin chain combinations, as the presence of an immunoglobulin light chain in only one half of the bispecific molecule provides for a facile way of separation. For further details of generating bispecific antibodies, see, for example, Suresh *et al.*, Methods in Enzymology 121:210 (1986).

According to another approach, the interface between a pair of antibody molecules can be engineered to maximize the percentage of heterodimers which are recovered from recombinant cell culture. The preferred interface comprises at least a part of the C_H3 domain of an antibody constant domain. In this method, one or more small amino acid side chains from the interface of the first antibody molecule are replaced with larger side chains (e.g. tyrosine or tryptophan). Compensatory "cavities" of identical or similar size to the large side chain(s) are created on the interface of the second antibody molecule by replacing large amino acid side chains with smaller ones (e.g. alanine or threonine). This provides a mechanism for increasing the yield of the heterodimer over other unwanted end-products such as

homodimers.

Bispecific antibodies include cross-linked or "heteroconjugate" antibodies. For example, one of the antibodies in the heteroconjugate can be coupled to avidin, the other to biotin. Such antibodies have, for example, been proposed to target immune system cells to unwanted cells (US Patent No. 4,676,980), and for treatment of HIV infection (WO 91/00360, WO 92/00373, and EP 03089). Heteroconjugate antibodies may be made using any convenient cross-linking methods. Suitable cross-linking agents are well known in the art, and are disclosed in US Patent No. 4,676,980, along with a number of cross-linking techniques.

Techniques for generating bispecific antibodies from antibody fragments have also been described in the literature. For example, bispecific antibodies can be prepared using chemical linkage. Brennan *et al.*, *Science*, 229: 81 (1985) describe a procedure wherein intact antibodies are proteolytically cleaved to generate F(ab')₂ fragments. These fragments are reduced in the presence of the dithiol complexing agent sodium arsenite to stabilize vicinal dithiols and prevent intermolecular disulfide formation. The Fab' fragments generated are then converted to thionitrobenzoate (TNB) derivatives. One of the Fab'-TNB derivatives is then reconverted to the Fab'-thiol by reduction with mercaptoethylamine and is mixed with an equimolar amount of the other Fab'-TNB derivative to form the bispecific antibody. The bispecific antibodies produced can be used as agents for the selective immobilization of enzymes.

Recent progress has facilitated the direct recovery of Fab'-SH fragments from *E. coli*, which can be chemically coupled to form bispecific antibodies. Shalaby *et al.*, *J. Exp. Med.*, 175: 217-225 (1992) describe the production of a fully humanized bispecific antibody F(ab')₂ molecule. Each Fab' fragment was separately secreted from *E. coli* and subjected to directed chemical coupling *in vitro* to form the bispecific antibody. The bispecific antibody thus formed was able to bind to cells overexpressing the HER2 receptor and normal human T cells, as well as trigger the lytic activity of human cytotoxic lymphocytes against human breast tumor targets.

Various techniques for making and isolating bispecific antibody fragments directly from recombinant cell culture have also been described. For example, bispecific antibodies have been produced using leucine zippers. Kostelny *et al.*, *J. Immunol.*, 148(5):1547-1553 (1992). The leucine zipper peptides from the Fos and Jun proteins were linked to the Fab' portions of two different antibodies by gene fusion. The antibody homodimers were reduced at the hinge region to form monomers and then re-oxidized to form the antibody heterodimers. This method can also be utilized for the production of antibody homodimers. The "diabody" technology described by Hollinger *et al.*, *Proc. Natl. Acad. Sci. USA*, 90:6444-6448 (1993) has provided an alternative mechanism for making bispecific antibody fragments. The fragments comprise a heavy-chain variable domain (VH) connected to a light-chain variable domain (VL) by a linker which is too short to allow pairing between the two domains on the same chain. Accordingly, the VH and VL domains of one fragment are forced to pair with the complementary VL and VH domains of another fragment, thereby forming two antigen-binding sites. Another strategy for making bispecific antibody fragments by the use of single-chain Fv (sFv) dimers has also been reported. See Gruber *et al.*, *J. Immunol.*, 152:5368 (1994).

Antibodies with more than two valencies are contemplated. For example, trispecific antibodies can be prepared. Tutt *et al.* *J. Immunol.* 147: 60 (1991).

4. Production of Humanized Anti-IL-8 6G4.2.5 Monoclonal Antibody, Antibody Fragments, and Variants

5 The antibodies and antibody fragments of the invention can be produced using any convenient antibody manufacturing process known in the art. Typically, the antibody or antibody fragment is made using recombinant expression systems. A multiple polypeptide chain antibody or antibody fragment species can be made in a single host cell expression system wherein the host cell produces each chain of the antibody or antibody fragment and assembles the polypeptide chains into a multimeric structure
10 to form the antibody or antibody fragment in vivo, followed by recovery of the antibody or antibody fragment from the host cell. For example, suitable recombinant expression systems for the production of complete antibody or antibody fragment are described in Lucas *et al.*, *Nucleic Acids Res.*, 24: 1774-1779 (1996). Alternatively, the separate polypeptide chains of the desired antibody or antibody fragment can be made in separate expression host cells, separately recovered from the respective host cells, and then mixed
15 in vitro under conditions permitting the formation of the multi-subunit antibody or antibody fragment of interest. For example, U.S. Pat. No. 4,816,567 to Cabilly *et al.* and Carter *et al.*, *Bio/Technology*, 10: 163-167 (1992) provide methods for recombinant production of antibody heavy and light chains in separate expression hosts followed by assembly of antibody from separate heavy and light chains in vitro.

The following discussion of recombinant expression methods applies equally to the production of
20 single chain antibody polypeptide species and multi-subunit antibody and antibody fragment species. All recombinant procedures for the production of antibody or antibody fragment provided below shall be understood to describe: (1) manufacture of single chain antibody species as the desired end-product; (2) manufacture of multi-subunit antibody or antibody fragment species by production of all subunits in a single host cell, subunit assembly in the host cell, optionally followed by host cell secretion of the multi-subunit
25 end-product into the culture medium, and recovery of the multi-subunit end-product from the host cell and/or culture medium; and (3) manufacture of multi-subunit antibody or antibody fragment by production of subunits in separate host cells (optionally followed by host cell secretion of subunits into the culture medium), recovery of subunits from the respective host cells and/or culture media, followed by in vitro subunit assembly to form the multi-subunit end-product. In the case of a multi-subunit antibody or antibody
30 fragment produced in a single host cell, it will be appreciated that production of the various subunits can be effected by expression of multiple polypeptide-encoding nucleic acid sequences carried on a single vector or by expression of polypeptide-encoding nucleic acid sequences carried on multiple vectors contained in the host cell.

A. Construction of DNA Encoding Humanized 6G4.2.5 Monoclonal Antibodies, Antibody Fragments, and Variants

Following the selection of the humanized antibody or antibody fragment of the invention according to the methods described above, the practitioner can use the genetic code to design DNAs

encoding the desired antibody or antibody fragment. In one embodiment, codons preferred by the expression host cell are used in the design of a DNA encoding the antibody or antibody fragment of interest. DNA encoding the desired antibody or antibody fragment can be prepared by a variety of methods known in the art. These methods include, but are not limited to, chemical synthesis by any of the methods described in Engels *et al.*, Angew. Chem. Int. Ed. Engl., **28**: 716-734 (1989), the entire disclosure of which is incorporated herein by reference, such as the triester, phosphite, phosphoramidite and H-phosphonate methods.

A variation on the above procedures contemplates the use of gene fusions, wherein the gene(s) encoding the antibody or antibody fragment is associated, in the vector, with a gene encoding another protein or a fragment of another protein. This results in the antibody or antibody fragment being produced by the host cell as a fusion with another protein. The "other" protein is often a protein or peptide which can be secreted by the cell, making it possible to isolate and purify the desired protein from the culture medium and eliminating the necessity of destroying the host cells which arises when the desired protein remains inside the cell. Alternatively, the fusion protein can be expressed intracellularly. It is advantageous to use fusion proteins that are highly expressed.

The use of gene fusions, though not essential, can facilitate the expression of heterologous proteins in *E. coli* as well as the subsequent purification of those gene products (Harris, T. J. R. in *Genetic Engineering*, Williamson, R., Ed., Academic, London, Vol. 4, p. 127(1983); Uhlen, M. & Moks, T., *Methods Enzymol.* **185**:129-143 (1990)). Protein A fusions are often used because the binding of protein A, or more specifically the Z domain of protein A, to IgG provides an "affinity handle" for the purification of the fused protein (Nilsson, B. & Abrahmsen, L. *Methods Enzymol.* **185**:144-161 (1990)). It has also been shown that many heterologous proteins are degraded when expressed directly in *E. coli*, but are stable when expressed as fusion proteins (Marston, F. A. O., *Biochem J.* **240**: 1 (1986)).

Fusion proteins can be cleaved using chemicals, such as cyanogen bromide, which cleaves at a methionine, or hydroxylamine, which cleaves between an Asn and Gly. Using standard recombinant DNA methodology, the nucleotide base pairs encoding these amino acids may be inserted just prior to the 5' end of the antibody or antibody fragment gene(s).

Alternatively, one can employ proteolytic cleavage of fusion proteins, which has been recently reviewed (Carter, P. (1990) in *Protein Purification: From Molecular Mechanisms to Large-Scale Processes*, Ladisch, M. R., Willson, R. C., Painton, C. C., and Builder, S. E., eds., American Chemical Society Symposium Series No. 427, Ch 13, 181-193).

Proteases such as Factor Xa, thrombin, subtilisin and mutants thereof, have been successfully used to cleave fusion proteins. Typically, a peptide linker that is amenable to cleavage by the protease used is inserted between the "other" protein (e.g., the Z domain of protein A) and the protein of interest, such as humanized anti-IL-8 antibody or antibody fragment. Using recombinant DNA methodology, the nucleotide base pairs encoding the linker are inserted between the genes or gene fragments coding for the other proteins. Proteolytic cleavage of the partially purified fusion protein containing the correct linker can then

be carried out on either the native fusion protein, or the reduced or denatured fusion protein.

Various techniques are also available which may now be employed to produce variant humanized antibodies or antibody fragments, which encodes for additions, deletions, or changes in amino acid sequence of the resultant protein(s) relative to the parent humanized antibody or antibody fragment.

5 By way of illustration, with expression vectors encoding humanized antibody or antibody fragment in hand, site specific mutagenesis (Kunkel *et al.*, *Methods Enzymol.* 204:125-139 (1991); Carter, P., *et al.*, *Nucl. Acids. Res.* 13:4331 (1986); Zoller, M. J. *et al.*, *Nucl. Acids Res.* 10:6487 (1982)), cassette mutagenesis (Wells, J. A., *et al.*, *Gene* 34:315 (1985)), restriction selection mutagenesis (Wells, J. A., *et al.*, *Philos. Trans, R. Soc. London SerA* 317, 415 (1986)) or other known techniques may be performed on the antibody
10 or antibody fragment DNA. The variant DNA can then be used in place of the parent DNA by insertion into the aforementioned expression vectors. Growth of host bacteria containing the expression vectors with the mutant DNA allows the production of variant humanized antibodies or antibody fragments, which can be isolated as described herein.

B. Insertion of DNA into a Cloning Vehicle

15 The DNA encoding the antibody or antibody fragment is inserted into a replicable vector for further cloning (amplification of the DNA) or for expression. Many vectors are available, and selection of the appropriate vector will depend on (1) whether it is to be used for DNA amplification or for DNA expression, (2) the size of the DNA to be inserted into the vector, and (3) the host cell to be transformed with the vector. Each vector contains various components depending on its function (amplification of DNA
20 or expression of DNA) and the host cell for which it is compatible. The vector components generally include, but are not limited to, one or more of the following: a signal sequence, an origin of replication, one or more marker genes, an enhancer element, a promoter, and a transcription termination sequence.

(i) Signal Sequence Component

25 In general, a signal sequence may be a component of the vector, or it may be a part of the antibody or antibody fragment DNA that is inserted into the vector. Preferably, a heterologous signal sequence selected and fused to the antibody or antibody fragment DNA such that the signal sequence in the corresponding fusion protein is recognized, transported and processed (*i.e.*, cleaved by a signal peptidase) in the host cell's protein secretion system. In the case of prokaryotic host cells, the signal sequence is selected, for example, from the group of the alkaline phosphatase, penicillinase, lpp, or heat-stable enterotoxin II leaders. In a preferred embodiment, the STII signal sequence is used as described in the
30 Examples below. For yeast secretion the native signal sequence may be substituted by, *e.g.*, the yeast invertase leader, α factor leader (including *Saccharomyces* and *Kluyveromyces* α -factor leaders), or acid phosphatase leader, the *C. albicans* glucoamylase leader, or the signal described in WO 90/13646. In mammalian cell expression, mammalian signal sequences as well as viral secretory leaders, for example, the
35 herpes simplex gD signal, are available.

(ii) Origin of Replication Component

Both expression and cloning vectors contain a nucleic acid sequence that enables

the vector to replicate in one or more selected host cells. Generally, in cloning vectors this sequence is one that enables the vector to replicate independently of the host chromosomal DNA, and includes origins of replication or autonomously replicating sequences. Such sequences are well known for a variety of bacteria, yeast, and viruses. The origin of replication from the plasmid pBR322 is suitable for most Gram-negative bacteria, the 2 μ plasmid origin is suitable for yeast, and various viral origins (SV40, polyoma, adenovirus, VSV or BPV) are useful for cloning vectors in mammalian cells. Generally, the origin of replication component is not needed for mammalian expression vectors (the SV40 origin may typically be used only because it contains the early promoter).

Most expression vectors are "shuttle" vectors, i.e. they are capable of replication in at least one class of organisms but can be transfected into another organism for expression. For example, a vector is cloned in *E. coli* and then the same vector is transfected into yeast or mammalian cells for expression even though it is not capable of replicating independently of the host cell chromosome.

DNA may also be amplified by insertion into the host genome. This is readily accomplished using *Bacillus* species as hosts, for example, by including in the vector a DNA sequence that is homologous to a sequence found in *Bacillus* genomic DNA. Transfection of *Bacillus* with this vector results in homologous recombination with the genome and insertion of the antibody or antibody fragment DNA.

(iii) Selection Gene Component

Expression and cloning vectors should contain a selection gene, also termed a selectable marker. This gene encodes a protein necessary for the survival or growth of transformed host cells grown in a selective culture medium. Host cells not transformed with the vector containing the selection gene will not survive in the culture medium. Typical selection genes encode proteins that (a) confer resistance to antibiotics or other toxins, e.g. ampicillin, neomycin, methotrexate, or tetracycline, (b) complement auxotrophic deficiencies, or (c) supply critical nutrients not available from complex media, e.g. the gene encoding D-alanine racemase for *Bacilli*.

One example of a selection scheme utilizes a drug to arrest growth of a host cell. Those cells that are successfully transformed with a heterologous gene express a protein conferring drug resistance and thus survive the selection regimen. Examples of such dominant selection use the drugs neomycin (Southern *et al.*, *J. Molec. Appl. Genet.*, 1: 327 (1982)), mycophenolic acid (Mulligan *et al.*, *Science*, 209: 1422 (1980)) or hygromycin (Sugden *et al.*, *Mol. Cell. Biol.*, 5: 410-413 (1985)). The three examples given above employ bacterial genes under eukaryotic control to convey resistance to the appropriate drug (G418 or neomycin (geneticin), xgpt (mycophenolic acid), and hygromycin, respectively.)

Another example of suitable selectable markers for mammalian cells are those that enable the identification of cells competent to take up the antibody or antibody fragment nucleic acid, such as dihydrofolate reductase (DHFR) or thymidine kinase. The mammalian cell transformants are placed under selection pressure which only the transformants are uniquely adapted to survive by virtue of having taken up the marker. Selection pressure is imposed by culturing the transformants under conditions in which the concentration of selection agent in the medium is successively changed, thereby leading to amplification of

both the selection gene and the DNA that encodes the antibody or antibody fragment. Amplification is the process by which genes in greater demand for the production of a protein critical for growth are reiterated in tandem within the chromosomes of successive generations of recombinant cells. Increased quantities of the antibody or antibody fragment are synthesized from the amplified DNA.

5 For example, cells transformed with the DHFR selection gene are first identified by culturing all of the transformants in a culture medium that contains methotrexate (Mtx), a competitive antagonist of DHFR. An appropriate host cell when wild-type DHFR is employed is the Chinese hamster ovary (CHO) cell line deficient in DHFR activity, prepared and propagated as described by Urlaub and Chasin, Proc. Natl. Acad. Sci. USA, 77: 4216 (1980). The transformed cells are then exposed to increased levels of methotrexate.
10 This leads to the synthesis of multiple copies of the DHFR gene, and, concomitantly, multiple copies of other DNA comprising the expression vectors, such as the DNA encoding the antibody or antibody fragment. This amplification technique can be used with any otherwise suitable host, e.g., ATCC No. CCL61 CHO-K1, notwithstanding the presence of endogenous DHFR if, for example, a mutant DHFR gene that is highly resistant to Mtx is employed (EP 117,060). Alternatively, host cells (particularly wild-type
15 hosts that contain endogenous DHFR) transformed or co-transformed with DNA sequences encoding the antibody or antibody fragment, wild-type DHFR protein, and another selectable marker such as aminoglycoside 3' phosphotransferase (APH) can be selected by cell growth in medium containing a selection agent for the selectable marker such as an aminoglycosidic antibiotic, e.g., kanamycin, neomycin, or G418. See U.S. Pat. No. 4,965,199.

20 A suitable selection gene for use in yeast is the *trp1* gene present in the yeast plasmid YRp7. Stinchcomb *et al.*, Nature, 282: 39 (1979); Kingsman *et al.*, Gene, 7: 141 (1979); or Tschemper *et al.*, Gene, 10: 157 (1980). The *trp1* gene provides a selection marker for a mutant strain of yeast lacking the ability to grow in tryptophan, for example, ATCC No. 44076 or PEP4-1. Jones, Genetics, 85: 12 (1977). The presence of the *trp1* lesion in the yeast host cell genome then provides an effective environment for
25 detecting transformation by growth in the absence of tryptophan. Similarly, *Leu2*-deficient yeast strains (ATCC 20,622 or 38,626) are complemented by known plasmids bearing the *Leu2* gene.

(iv) Promoter Component

Expression vectors usually contain a promoter that is recognized by the host organism and is operably linked to the antibody or antibody fragment nucleic acid. Promoters are
30 untranslated sequences located upstream (5') to the start codon of a structural gene (generally within about 100 to 1000 bp) that control the transcription and translation of a particular nucleic acid sequence, such as the antibody or antibody fragment encoding sequence, to which they are operably linked. Such promoters typically fall into two classes, inducible and constitutive. Inducible promoters are promoters that initiate increased levels of transcription from DNA under their control in response to some change in culture
35 conditions, e.g. the presence or absence of a nutrient or a change in temperature. At this time a large number of promoters recognized by a variety of potential host cells are well known.

Promoters suitable for use with prokaryotic hosts include the β -lactamase and lactose promoter

systems (Chang *et al.*, Nature, 275: 615 (1978); and Goeddel *et al.*, Nature, 281: 544 (1979)), alkaline phosphatase, a tryptophan (trp) promoter system (Goeddel, Nucleic Acids Res., 8: 4057 (1980) and EP 36,776) and hybrid promoters such as the tac promoter (deBoer *et al.*, Proc. Natl. Acad. Sci. USA, 80: 21-25 (1983)). However, other known bacterial promoters are suitable. Their nucleotide sequences have been published, thereby enabling a skilled worker to operably ligate them to DNA encoding the antibody or antibody fragment (Siebenlist *et al.*, Cell, 20: 269 (1980)) using linkers or adaptors to supply any required restriction sites. Promoters for use in bacterial systems also generally will contain a Shine-Dalgarno (S.D.) sequence operably linked to the DNA encoding the antibody or antibody fragment.

Suitable promoting sequences for use with yeast hosts include the promoters for 3-phosphoglycerate kinase (Hitzeman *et al.*, J. Biol. Chem., 255: 2073 (1980)) or other glycolytic enzymes (Hess *et al.*, J. Adv. Enzyme Reg., 7: 149 (1968); and Holland, Biochemistry, 17: 4900 (1978)), such as enolase, glyceraldehyde-3-phosphate dehydrogenase, hexokinase, pyruvate decarboxylase, phosphofructokinase, glucose-6-phosphate isomerase, 3-phosphoglycerate mutase, pyruvate kinase, triosephosphate isomerase, phosphoglucose isomerase, and glucokinase.

Other yeast promoters, which are inducible promoters having the additional advantage of transcription controlled by growth conditions, are the promoter regions for alcohol dehydrogenase 2, isocytochrome C, acid phosphatase, degradative enzymes associated with nitrogen metabolism, metallothionein, glyceraldehyde-3-phosphate dehydrogenase, and enzymes responsible for maltose and galactose utilization. Suitable vectors and promoters for use in yeast expression are further described in Hitzeman *et al.*, EP 73,657A. Yeast enhancers also are advantageously used with yeast promoters.

Promoter sequences are known for eukaryotes. Virtually all eukaryotic genes have an AT-rich region located approximately 25 to 30 bases upstream from the site where transcription is initiated. Another sequence found 70 to 80 bases upstream from the start of transcription of many genes is a CXCAAT region where X may be any nucleotide. At the 3' end of most eukaryotic genes is an AATAAA sequence that may be the signal for addition of the poly A tail to the 3' end of the coding sequence. All of these sequences are suitably inserted into mammalian expression vectors.

Vector driven transcription of antibody or antibody fragment encoding DNA in mammalian host cells can be controlled by promoters obtained from the genomes of viruses such as polyoma virus, fowlpox virus (UK 2,211,504 published 5 July 1989), adenovirus (such as Adenovirus 2), bovine papilloma virus, avian sarcoma virus, cytomegalovirus, a retrovirus, hepatitis-B virus and most preferably Simian Virus 40 (SV40), from heterologous mammalian promoters, e.g. the actin promoter or an immunoglobulin promoter, and from heat-shock promoters, provided such promoters are compatible with the host cell systems.

The early and late promoters of the SV40 virus are conveniently obtained as an SV40 restriction fragment that also contains the SV40 viral origin of replication. Fiers *et al.*, Nature, 273: 113 (1978); Mulligan and Berg, Science, 209: 1422-1427 (1980); Pavlakis *et al.*, Proc. Natl. Acad. Sci. USA, 78: 7398-7402 (1981). The immediate early promoter of the human cytomegalovirus is conveniently obtained as a HindIII E restriction fragment. Greenaway *et al.*, Gene, 18: 355-360 (1982). A system for expressing DNA

in mammalian hosts using the bovine papilloma virus as a vector is disclosed in U.S. 4,419,446. A modification of this system is described in U.S. 4,601,978. See also Gray *et al.*, Nature, 295: 503-508 (1982) on expressing cDNA encoding immune interferon in monkey cells, Reyes *et al.*, Nature, 297: 598-601 (1982) on expression of human γ -interferon cDNA in mouse cells under the control of a thymidine kinase promoter from herpes simplex virus, Canaani and Berg, Proc. Natl. Acad. Sci. USA, 79: 5166-5170 (1982) on expression of the human interferon β gene in cultured mouse and rabbit cells, and Gorman *et al.*, Proc. Natl. Acad. Sci. USA, 79: 6777-6781 (1982) on expression of bacterial CAT sequences in CV-1 monkey kidney cells, chicken embryo fibroblasts, Chinese hamster ovary cells, HeLa cells, and mouse NIH-3T3 cells using the Rous sarcoma virus long terminal repeat as a promoter.

10 (v) Enhancer Element Component

Transcription of a DNA encoding antibody or antibody fragment by higher eukaryotic host cells is often increased by inserting an enhancer sequence into the vector. Enhancers are cis-acting elements of DNA, usually about from 10-300 bp, that act on a promoter to increase its transcription. Enhancers are relatively orientation and position independent having been found 5' (Laimins *et al.*, Proc. Natl. Acad. Sci. USA, 78: 993 (1981)) and 3' (Lusky *et al.*, Mol. Cell Bio., 3: 1108 (1983)) to the transcription unit, within an intron (Banerji *et al.*, Cell, 33: 729 (1983)) as well as within the coding sequence itself (Osborne *et al.*, Mol. Cell Bio., 4: 1293 (1984)). Many enhancer sequences are now known from mammalian genes (globin, elastase, albumin, α -fetoprotein and insulin). Typically, however, one will use an enhancer from a eukaryotic cell virus. Examples include the SV40 enhancer on the late side of the replication origin (bp 100-270), the cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers. See also Yaniv, Nature, 297: 17-18 (1982) on enhancing elements for activation of eukaryotic promoters. The enhancer may be spliced into the vector at a position 5' or 3' to the antibody or antibody fragment DNA, but is preferably located at a site 5' from the promoter.

25 (vi) Transcription Termination Component

Expression vectors used in eukaryotic host cells (yeast, fungi, insect, plant, animal, human, or nucleated cells from other multicellular organisms) can also contain sequences necessary for the termination of transcription and for stabilizing the mRNA. Such sequences are commonly available from the 5' and, occasionally 3' untranslated regions of eukaryotic or viral DNAs or cDNAs. These regions contain nucleotide segments transcribed as polyadenylated fragments in the untranslated portion of the mRNA encoding the antibody or antibody fragment. The 3' untranslated regions also include transcription termination sites.

Suitable vectors containing one or more of the above listed components and the desired coding and control sequences are constructed by standard ligation techniques. Isolated plasmids or DNA fragments are cleaved, tailored, and religated in the form desired to generate the plasmids required.

For analysis to confirm correct sequences in plasmids constructed, the ligation mixtures are used to transform *E. coli* K12 strain 294 (ATCC 31,446) and successful transformants selected by ampicillin or

tetracycline resistance where appropriate. Plasmids from the transformants are prepared, analyzed by restriction endonuclease digestion, and/or sequenced by the method of Messing *et al.*, Nucleic Acids Res., **9**: 309 (1981) or by the method of Maxam *et al.*, Methods in Enzymology, **65**: 499 (1980).

5 Particularly useful in the practice of this invention are expression vectors that provide for the transient expression in mammalian cells of DNA encoding the antibody or antibody fragment. In general, transient expression involves the use of an expression vector that is able to replicate efficiently in a host cell, such that the host cell accumulates many copies of the expression vector and, in turn, synthesizes high levels of a desired polypeptide encoded by the expression vector.

10 Other methods, vectors, and host cells suitable for adaptation to the synthesis of the antibody or antibody fragment in recombinant vertebrate cell culture are described in Gething *et al.*, Nature, **293**: 620-625 (1981); Mantei *et al.*, Nature, **281**: 40-46 (1979); Levinson *et al.*, EP 117,060; and EP 117,058. A particularly useful plasmid for mammalian cell culture expression of the IgE peptide antagonist is pRK5 (EP pub. no. 307,247) or pSV16B (PCT pub. no. WO 91/08291 published 13 June 1991).

C. Selection and Transformation of Host Cells

15 Suitable host cells for cloning or expressing the vectors herein are the prokaryote, yeast, or higher eukaryote cells described above. Suitable prokaryotes include eubacteria, such as Gram-negative or Gram-positive organisms, for example, *E. coli*, *Bacilli* such as *B. subtilis*, *Pseudomonas* species such as *P. aeruginosa*, *Salmonella typhimurium*, or *Serratia marcescens*. One preferred *E. coli* cloning host is *E. coli* 294 (ATCC 31,446), although other strains such as *E. coli* B, *E. coli* 1776 (ATCC 31,537), and *E. coli* 20 W3110 (ATCC 27,325) are suitable. These examples are illustrative rather than limiting. Preferably the host cell should secrete minimal amounts of proteolytic enzymes. In a preferred embodiment, the *E. coli* strain 49D6 is used as the expression host as described in the Examples below. Review articles describing the recombinant production of antibodies in bacterial host cells include Skerra *et al.*, Curr. Opinion in Immunol., **5**: 256 (1993) and Pluckthun, Immunol. Revs., **130**: 151 (1992).

25 In addition to prokaryotes, eukaryotic microbes such as filamentous fungi or yeast are suitable hosts for vectors containing antibody or antibody fragment DNA. *Saccharomyces cerevisiae*, or common baker's yeast, is the most commonly used among lower eukaryotic host microorganisms. However, a number of other genera, species, and strains are commonly available and useful herein, such as *S. pombe* (Beach and Nurse, Nature, **290**: 140 (1981)), *Kluyveromyces lactis* (Louvencourt *et al.*, J. Bacteriol., **737** (1983)), *Yarrowia* (EP 402,226), *Pichia pastoris* (EP 183,070), *Trichoderma reesia* (EP 244,234), 30 *Neurospora crassa* (Case *et al.*, Proc. Natl. Acad. Sci. USA, **76**: 5259-5263 (1979)), and *Aspergillus* hosts such as *A. nidulans* (Ballance *et al.*, Biochem. Biophys. Res. Commun., **112**: 284-289 (1983); Tilburn *et al.*, Gene, **26**: 205-221 (1983); Yelton *et al.*, Proc. Natl. Acad. Sci. USA, **81**: 1470-1474 (1984)) and *A. niger* (Kelly and Hynes, EMBO J., **4**: 475-479 (1985)).

35 Host cells derived from multicellular organisms can also be used in the recombinant production of antibody or antibody fragment. Such host cells are capable of complex processing and glycosylation activities. In principle, any higher eukaryotic cell culture is workable, whether from vertebrate or

invertebrate culture. Examples of invertebrate cells include plant and insect cells. Numerous baculoviral strains and variants and corresponding permissive insect host cells from hosts such as *Spodoptera frugiperda* (caterpillar), *Aedes aegypti* (mosquito), *Aedes albopictus* (mosquito), *Drosophila melanogaster* (fruitfly), and *Bombyx mori* host cells have been identified. See, e.g., Luckow *et al.*, Bio/Technology, **6**: 47-55 (1988); Miller *et al.*, in Genetic Engineering, Setlow, J.K. *et al.*, **8**: 277-279 (Plenum Publishing, 1986), and Maeda *et al.*, Nature, **315**: 592-594 (1985). A variety of such viral strains are publicly available, e.g., the L-1 variant of *Autographa californica* NPV and the Bm-5 strain of *Bombyx mori* NPV, and such viruses may be used as the virus herein according to the present invention, particularly for transfection of *Spodoptera frugiperda* cells.

Plant cell cultures of cotton, corn, potato, soybean, petunia, tomato, and tobacco can be utilized as hosts. Typically, plant cells are transfected by incubation with certain strains of the bacterium *Agrobacterium tumefaciens*, which has been previously manipulated to contain the antibody or antibody fragment DNA. During incubation of the plant cell culture with *A. tumefaciens*, the DNA encoding antibody or antibody fragment is transferred to the plant cell host such that it is transfected, and will, under appropriate conditions, express the antibody or antibody fragment DNA. In addition, regulatory and signal sequences compatible with plant cells are available, such as the nopaline synthase promoter and polyadenylation signal sequences. Depicker *et al.*, J. Mol. Appl. Gen., **1**: 561 (1982). In addition, DNA segments isolated from the upstream region of the T-DNA 780 gene are capable of activating or increasing transcription levels of plant-expressible genes in recombinant DNA-containing plant tissue. See EP 321,196 published 21 June 1989.

Vertebrate cell culture is preferred for the recombinant production of full length antibodies. The propagation of vertebrate cells in culture (tissue culture) has become a routine procedure in recent years (Tissue Culture, Academic Press, Kruse and Patterson, editors (1973)). Examples of useful mammalian host cell lines are monkey kidney CV1 line transformed by SV40 (COS-7, ATCC CRL 1651); human embryonic kidney line (293 or 293 cells subcloned for growth in suspension culture, Graham *et al.*, J. Gen Virol., **36**: 59 (1977)); baby hamster kidney cells (BHK, ATCC CCL 10); Chinese hamster ovary cells/-DHFR (CHO, Urlaub and Chasin, Proc. Natl. Acad. Sci. USA, **77**: 4216 (1980)); mouse sertoli cells (TM4, Mather, Biol. Reprod., **23**: 243-251 (1980)); monkey kidney cells (CV1 ATCC CCL 70); African green monkey kidney cells (VERO-76, ATCC CRL-1587); human cervical carcinoma cells (HELA, ATCC CCL 2); canine kidney cells (MDCK, ATCC CCL 34); buffalo rat liver cells (BRL 3A, ATCC CRL 1442); human lung cells (W138, ATCC CCL 75); human liver cells (Hep G2, HB 8065); mouse mammary tumor (MMT 060562, ATCC CCL51); TRI cells (Mather *et al.*, Annals N.Y. Acad. Sci., **383**: 44-68 (1982)); MRC 5 cells; FS4 cells; and a human hepatoma cell line (Hep G2). Preferred host cells are human embryonic kidney 293 and Chinese hamster ovary cells. Myeloma cells that do not otherwise produce immunoglobulin protein are also useful host cells for the recombinant production of full length antibodies.

Host cells are transfected and preferably transformed with the above-described expression or cloning vectors of this invention and cultured in conventional nutrient media modified as appropriate for

inducing promoters, selecting transformants, or amplifying the genes encoding the desired sequences.

Transfection refers to the taking up of an expression vector by a host cell whether or not any coding sequences are in fact expressed. Numerous methods of transfection are known to the ordinarily skilled artisan, for example, CaPO_4 precipitation and electroporation. Successful transfection is generally
5 recognized when any indication of the operation of this vector occurs within the host cell.

Transformation means introducing DNA into an organism so that the DNA is replicable, either as an extrachromosomal element or by chromosomal integrant. Depending on the host cell used, transformation is done using standard techniques appropriate to such cells. The calcium treatment employing calcium chloride, as described in section 1.82 of Sambrook *et al.*, *supra*, is generally used for
10 prokaryotes or other cells that contain substantial cell-wall barriers. Infection with *Agrobacterium tumefaciens* is used for transformation of certain plant cells, as described by Shaw *et al.*, Gene, 23: 315 (1983) and WO 89/05859 published 29 June 1989. For mammalian cells without such cell walls, the calcium phosphate precipitation method described in sections 16.30-16.37 of Sambrook *et al.*, *supra*, is preferred. General aspects of mammalian cell host system transformations have been described by Axel in
15 U.S. 4,399,216 issued 16 August 1983. Transformations into yeast are typically carried out according to the method of Van Solingen *et al.*, J. Bact., 130: 946 (1977) and Hsiao *et al.*, Proc. Natl. Acad. Sci. (USA), 76: 3829 (1979). However, other methods for introducing DNA into cells such as by nuclear injection, electroporation, or by protoplast fusion may also be used.

D. Culturing the Host Cells

20 Prokaryotic cells used to produce the antibody or antibody fragment are cultured in suitable media as described generally in Sambrook *et al.*, *supra*.

The mammalian host cells used to produce the antibody or antibody fragment can be cultured in a variety of media. Commercially available media such as Ham's F10 (Sigma), Minimal Essential Medium ((MEM), Sigma), RPMI-1640 (Sigma), and Dulbecco's Modified Eagle's Medium ((DMEM), Sigma) are
25 suitable for culturing the host cells. In addition, any of the media described in Ham and Wallace, Meth. Enz., 58: 44 (1979), Barnes and Sato, Anal. Biochem., 102: 255 (1980), U.S. 4,767,704; 4,657,866; 4,927,762; or 4,560,655; WO 90/03430; WO-87/00195; U.S. Pat. Re. 30,985; or U.S. 5,122,469, the disclosures of all of which are incorporated herein by reference, may be used as culture media for the host cells. Any of these media may be supplemented as necessary with hormones and/or other growth factors
30 (such as insulin, transferrin, or epidermal growth factor), salts (such as sodium chloride, calcium, magnesium, and phosphate), buffers (such as HEPES), nucleosides (such as adenosine and thymidine), antibiotics (such as GentamycinTM drug), trace elements (defined as inorganic compounds usually present at final concentrations in the micromolar range), and glucose or an equivalent energy source. Any other necessary supplements may also be included at appropriate concentrations that would be known to those
35 skilled in the art. The culture conditions, such as temperature, pH, and the like, are those previously used with the host cell selected for expression, and will be apparent to the ordinarily skilled artisan.

The host cells referred to in this disclosure encompass cells in *in vitro* culture as well as cells that

are within a host animal.

E. Detecting Gene Amplification/Expression

Gene amplification and/or expression may be measured in a sample directly, for example, by conventional Southern blotting, northern blotting to quantitate the transcription of mRNA (Thomas, Proc. Natl. Acad. Sci. USA, 77: 5201-5205 (1980)), dot blotting (DNA analysis), or *in situ* hybridization, using an appropriately labeled probe, based on the sequences provided herein. Various labels may be employed, most commonly radioisotopes, particularly ^{32}P . However, other techniques may also be employed, such as using biotin-modified nucleotides for introduction into a polynucleotide. The biotin then serves as the site for binding to avidin or antibodies, which may be labeled with a wide variety of labels, such as radionuclides, fluorescers, enzymes, or the like. Alternatively, antibodies may be employed that can recognize specific duplexes, including DNA duplexes, RNA duplexes, and DNA-RNA hybrid duplexes or DNA-protein duplexes. The antibodies in turn may be labeled and the assay may be carried out where the duplex is bound to a surface, so that upon the formation of duplex on the surface, the presence of antibody bound to the duplex can be detected.

Gene expression, alternatively, may be measured by immunological methods, such as immunohistochemical staining of tissue sections and assay of cell culture or body fluids, to quantitate directly the expression of gene product. With immunohistochemical staining techniques, a cell sample is prepared, typically by dehydration and fixation, followed by reaction with labeled antibodies specific for the gene product, where the labels are usually visually detectable, such as enzymatic labels, fluorescent labels, luminescent labels, and the like. A particularly sensitive staining technique suitable for use in the present invention is described by Hsu *et al.*, Am. J. Clin. Path., 75: 734-738 (1980).

F. Purification of the Antibody or Antibody Fragment

In the case of a host cell secretion system, the antibody or antibody fragment is recovered from the culture medium. Alternatively, the antibody can be produced intracellularly, or produced in the periplasmic space of a bacterial host cell. If the antibody is produced intracellularly, as a first step, the host cells are lysed, and the resulting particulate debris is removed, for example, by centrifugation or ultrafiltration. Carter *et al.*, BioTechnology 10:163-167 (1992) describe a procedure for isolating antibodies which are secreted to the periplasmic space of *E. coli*. Briefly, cell paste is thawed in the presence of sodium acetate (pH 3.5), EDTA, and phenylmethylsulfonylfluoride (PMSF) over about 30 min. Cell debris can be removed by centrifugation. Where the antibody is secreted into the medium, supernatants from such expression systems are generally first concentrated using a commercially available protein concentration filter, for example, an Amicon or Millipore Pellicon ultrafiltration unit. A protease inhibitor such as PMSF may be included in any of the foregoing steps to inhibit proteolysis and antibiotics may be included to prevent the growth of adventitious contaminants.

The antibody composition prepared from the cells can be purified using, for example, hydroxylapatite chromatography, gel electrophoresis, dialysis, and affinity chromatography, with affinity chromatography being the preferred purification technique. The suitability of protein A as an affinity ligand

depends on the species and isotype of any immunoglobulin Fc domain that is present in the antibody. Protein A can be used to purify antibodies that are based on human $\gamma 1$, $\gamma 2$, or $\gamma 4$ heavy chains (Lindmark *et al.*, *J. Immunol. Meth.* 62:1-13 (1983)). Protein G is recommended for all mouse isotypes and for human $\gamma 3$ (Guss *et al.*, *EMBO J.* 5:1567-1575 (1986)). The matrix to which the affinity ligand is attached is most often agarose, but other matrices are available. Mechanically stable matrices such as controlled pore glass or poly(styrenedivinyl)benzene allow for faster flow rates and shorter processing times than can be achieved with agarose. Where the antibody comprises a C_{H3} domain, the Bakerbond ABXTM resin (J. T. Baker, Phillipsburg, NJ) is useful for purification. Other techniques for protein purification such as fractionation on an ion-exchange column, ethanol precipitation, Reverse Phase HPLC, chromatography on silica, chromatography on heparin SepharoseTM, chromatography on an anion or cation exchange resin (such as a polyaspartic acid column), chromatofocusing, SDS-PAGE, and ammonium sulfate precipitation are also available depending on the antibody to be recovered.

Following any preliminary purification step(s), the mixture comprising the antibody of interest and contaminants may be subjected to low pH hydrophobic interaction chromatography using an elution buffer at a pH between about 2.5-4.5, preferably performed at low salt concentrations (e.g. from about 0-0.25M salt).

G. Production of Antibody Fragments

Various techniques have been developed for the production of the humanized antibody fragments of the invention, including Fab, Fab', Fab'-SH, or F(ab')₂ fragments. Traditionally, these fragments were derived via proteolytic digestion of intact antibodies (see, e.g., Morimoto *et al.*, *Journal of Biochemical and Biophysical Methods* 24:107-117 (1992) and Brennan *et al.*, *Science*, 229:81 (1985)). However, these fragments can now be produced directly by recombinant host cells. For example, Fab'-SH fragments can be directly recovered from *E. coli* and chemically coupled to form F(ab')₂ fragments (Carter *et al.*, *Bio/Technology*, 10:163-167 (1992)). According to another approach, F(ab')₂ fragments can be isolated directly from recombinant host cell culture. Other techniques for the production of antibody fragments will be apparent to the skilled practitioner.

5. Uses of Anti-IL-8 Antibodies

A. Diagnostic Uses

For diagnostic applications requiring the detection or quantitation of IL-8, the antibodies or antibody fragments of the invention typically will be labeled with a detectable moiety. The detectable moiety can be any one which is capable of producing, either directly or indirectly, a detectable signal. For example, the detectable moiety can be a radioisotope, such as ³H, ¹⁴C, ³²P, ³⁵S, or ¹²⁵I; a fluorescent or chemiluminescent compound, such as fluorescein isothiocyanate, rhodamine, or luciferin; radioactive isotopic labels, such as, e.g., ¹²⁵I, ³²P, ¹⁴C, or ³H; or an enzyme, such as alkaline phosphatase, beta-galactosidase, or horseradish peroxidase.

Any method known in the art for separately conjugating the antibody or antibody fragment to the detectable moiety can be employed, including those methods described by Hunter *et al.*, Nature 144:945 (1962); David *et al.*, Biochemistry 13:1014 (1974); Pain *et al.*, J. Immunol. Meth. 40:219 (1981); and Nygren, J. Histochem. and Cytochem. 30:407 (1982).

5 The antibodies and antibody fragments of the present invention can be employed in any known assay method, such as competitive binding assays, direct and indirect sandwich assays, and immunoprecipitation assays. For example, see Zola, Monoclonal Antibodies: A Manual of Techniques, pp. 147-158 (CRC Press, Inc., 1987).

10 Competitive binding assays rely on the ability of a labeled standard (which can be a IL-8 or an immunologically reactive portion thereof) to compete with the test sample analyte (IL-8) for binding with a limited amount of antibody or antibody fragment. The amount of IL-8 in the test sample is inversely proportional to the amount of standard that becomes bound to the antibodies. To facilitate determining the amount of standard that becomes bound, the antibodies or antibody fragments generally are insolubilized before or after the competition, so that the standard and analyte that are bound to the antibodies can
15 conveniently be separated from the standard and analyte which remain unbound.

Sandwich assays involve the use of two antibodies, each capable of binding to a different antigenic portion, or epitope, of the protein (IL-8) to be detected. In a sandwich assay, the test sample analyte is bound by a first antibody which is immobilized on a solid support, and thereafter a second antibody binds to the analyte, thus forming an insoluble three-part complex (U.S. Patent No. 4,376,110). The second antibody
20 can itself be labeled with a detectable moiety (direct sandwich assays) or can be measured using an anti-immunoglobulin antibody that is labeled with a detectable moiety (indirect sandwich assay). For example, one type of sandwich assay is an ELISA assay, in which case the detectable moiety is an enzyme (e.g., horseradish peroxidase).

IL-8 antibodies and antibody fragments also are useful for the affinity purification of IL-8 from
25 recombinant cell culture or natural sources. For example, these antibodies can be fixed to a solid support by techniques well known in the art so as to purify IL-8 from a source such as culture supernatant or tissue.

B. Therapeutic Compositions and Administration of Anti-IL-8 Antibody

The humanized anti-IL-8 antibodies and antibody fragments of the invention are useful in the treatment of inflammatory disorders, such as adult respiratory distress syndrome (ARDS), hypovolemic
30 shock, ulcerative colitis, and rheumatoid arthritis.

Therapeutic formulations of the humanized anti-IL-8 antibodies and antibody fragments are prepared for storage by mixing the antibody or antibody fragment having the desired degree of purity with optional physiologically acceptable carriers, excipients, or stabilizers (Remington's Pharmaceutical Sciences, supra), in the form of lyophilized cake or aqueous solutions. Acceptable carriers, excipients or
35 stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid; low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins;

hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, arginine or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrans; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; salt-forming counterions such as sodium; and/or nonionic surfactants such as Tween, Pluronic or polyethylene glycol (PEG).

The humanized anti-IL-8 mAb or antibody fragment to be used for in vivo administration must be sterile. This is readily accomplished by filtration through sterile filtration membranes, prior to or following lyophilization and reconstitution. The humanized anti-IL-8 mAb or antibody fragment ordinarily will be stored in lyophilized form or in solution.

Therapeutic humanized anti-IL-8 mAb or antibody fragment compositions generally are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

The route of humanized anti-IL-8 mAb or antibody fragment administration is in accord with known methods, e.g., inhalation, injection or infusion by intravenous, intraperitoneal, intracerebral, intramuscular, intraocular, intraarterial, or intralesional routes, by enema or suppository, or by sustained release systems as noted below. Preferably the antibody is given systemically or at a site of inflammation.

Suitable examples of sustained-release preparations include semipermeable polymer matrices in the form of shaped articles, e.g. films, or microcapsules. Sustained release matrices include polyesters, hydrogels, polylactides (U.S. 3,773,919, EP 58,481), copolymers of L-glutamic acid and gamma ethyl-L-glutamate (Sidman *et al.*, Biopolymers 22:547 (1983)), poly (2-hydroxyethyl-methacrylate) (Langer *et al.*, J. Biomed. Mater. Res. 15:167 (1981) and Langer, Chem. Tech. 12:98 (1982)), ethylene vinyl acetate (Langer *et al.*, *supra*) or poly-D-(-)-3-hydroxybutyric acid (EP 133,988). Sustained-release humanized anti-IL-8 antibody or antibody fragment compositions also include liposomally entrapped antibody or antibody fragment. Liposomes containing an antibody or antibody fragment are prepared by methods known per se: DE 3,218,121; Epstein *et al.*, Proc. Natl. Acad. Sci. U.S.A. 82:3688 (1985); Hwang *et al.*, Proc. Natl. Acad. Sci. U.S.A. 77:4030 (1980); EP 52,322; EP 36,676; EP 88,046; EP 143,949; EP 142,641; Japanese patent application 83-118008; U.S. Patent Nos. 4,485,045 and 4,544,545; and EP 102,324. Ordinarily the liposomes are of the small (about 200-800 Angstroms) unilamellar type in which the lipid content is greater than about 30 mole percent cholesterol, the selected proportion being adjusted for the most efficacious antibody or antibody fragment therapy.

An "effective amount" of the humanized anti-IL-8 antibody or antibody fragment to be employed therapeutically will depend, for example, upon the therapeutic objectives, the route of administration, and the condition of the patient. Accordingly, it will be necessary for the therapist to titer the dosage and modify the route of administration as required to obtain the optimal therapeutic effect. Typically, the clinician will administer the humanized anti-IL-8 antibody or antibody fragment until a dosage is reached that achieves the desired effect. The progress of this therapy is easily monitored by conventional assays.

In the treatment and prevention of an inflammatory disorder with a humanized anti-IL-8 antibody

or antibody fragment of the invention, the antibody composition will be formulated, dosed, and administered in a fashion consistent with good medical practice. Factors for consideration in this context include the particular disorder being treated, the particular mammal being treated, the clinical condition of the individual patient, the cause of the disorder, the site of delivery of the antibody, the particular type of antibody, the method of administration, the scheduling of administration, and other factors known to medical practitioners. The "therapeutically effective amount" of antibody to be administered will be governed by such considerations, and is the minimum amount necessary to prevent, ameliorate, or treat the inflammatory disorder, including treating acute or chronic respiratory diseases and reducing inflammatory responses. Such amount is preferably below the amount that is toxic to the host or renders the host significantly more susceptible to infections.

As a general proposition, the initial pharmaceutically effective amount of the antibody or antibody fragment administered parenterally per dose will be in the range of about 0.1 to 50 mg/kg of patient body weight per day, with the typical initial range of antibody used being 0.3 to 20 mg/kg/day, more preferably 0.3 to 15 mg/kg/day.

As noted above, however, these suggested amounts of antibody or antibody fragment are subject to a great deal of therapeutic discretion. The key factor in selecting an appropriate dose and scheduling is the result obtained, as indicated above.

The antibody or antibody fragment need not be, but is optionally formulated with one or more agents currently used to prevent or treat the inflammatory disorder in question. For example, in rheumatoid arthritis, the antibody can be given in conjunction with a glucocorticosteroid. The effective amount of such other agents depends on the amount of antibody or antibody fragment present in the formulation, the type of disorder or treatment, and other factors discussed above. These are generally used in the same dosages and with administration routes as used hereinbefore or about from 1 to 99% of the heretofore employed dosages.

The following examples are offered by way of illustration and not by way of limitation. The disclosures of all references cited in the specification, and the disclosures of all citations in such references, are expressly incorporated herein by reference.

EXAMPLES

A. GENERATION AND CHARACTERIZATION OF MONOCLONAL ANTIBODIES AGAINST HUMAN IL-8

Balb/c mice were immunized in each hind footpad or intraperitoneally with 10 µg of recombinant human IL-8 (produced as a fusion of (ser-IL-8)₇₂ with ubiquitin (Hebert *et al.* J. Immunology 145:3033-3040 (1990)); IL-8 is available commercially from PeproTech, Inc., Rocky Hill, NJ) resuspended in MPL/TDM (Ribi Immunochem. Research Inc., Hamilton, MT) and boosted twice with the same amount of IL-8. In these experiments, "IL-8" is intended to mean (ser-IL-8)₇₂ unless otherwise specified. A final boost of 10 µg of IL-8 was given 3 days before the fusion. Spleen cells or popliteal lymph node cells were fused with mouse myeloma P3X63Ag8U.1 (ATCC CRL1597), a non-secreting clone of the myeloma P3X63Ag8, using 35% polyethylene glycol as described *supra*. Ten days after the fusion, culture supernatant was

screened for the presence of monoclonal antibodies to IL-8 by ELISA.

The ELISA was performed as follows. Nunc 96-well immunoplates (Flow Lab, McLean, VA) were coated with 50 µl/well of 2 µg/ml IL-8 in phosphate-buffered saline (PBS) overnight at 4°C. The remaining steps were carried out at room temperature. Nonspecific binding sites were blocked with 0.5% bovine serum albumin (BSA) for 1 hour (hr). Plates were then incubated with 50 µl/well of hybridoma culture supernatants from 672 growing parental fusion wells for 1 hr, followed by the incubation with 50 µl/well of 1:1000 dilution of a 1 mg/ml stock solution of alkaline phosphatase-conjugated goat anti-mouse Ig (Tago Co., Foster City, CA) for 1 hr. The level of enzyme-linked antibody bound to the plate was determined by the addition of 100 µl/well of 0.5 mg/ml of p-nitrophenyl phosphate in sodium bicarbonate buffer, pH 9.6. The color reaction was measured at 405 nm with an ELISA plate reader (Titertek Multiscan, Flow Lab, McLean, VA). Between each step, plates were washed three times in PBS containing 0.05% Tween 20.

Culture supernatants which promoted 4-fold more binding of IL-8 than did control medium were selected as positives. According to this criterion, 16 of 672 growing parental fusion wells (2%) were positive. These positive hybridoma cell lines were cloned at least twice by using the limiting dilution technique.

Seven of the positive hybridomas were further characterized as follows. The isotypes of the monoclonal antibodies were determined by coating Nunc 96-well immunoplates (Flow Lab, McLean, VA) with IL-8 overnight, blocking with BSA, incubating with culture supernatants followed by the addition of predetermined amount of isotype-specific alkaline phosphatase-conjugated goat anti-mouse Ig (Fisher Biotech, Pittsburgh, PA). The level of conjugated antibodies bound to the plate was determined by the addition of p-nitrophenyl phosphate as described above.

All the monoclonal antibodies tested belonged to either IgG₁ or IgG₂ immunoglobulin isotype. Ascites fluid containing these monoclonal antibodies had antibody titers in the range of 10,000 to 100,000 as determined by the reciprocal of the dilution factor which gave 50% of the maximum binding in the ELISA.

To assess whether these monoclonal antibodies bound to the same epitopes, a competitive binding ELISA was performed. At a ratio of biotinylated mAb to unlabeled mAb of 1:100, the binding of biotinylated mAb 5.12.14 was significantly inhibited by its homologous mAb but not by mAb 4.1.3, while the binding of biotinylated mAb 4.1.3 was inhibited by mAb 4.1.3 but not by mAb 5.12.14. Monoclonal antibody 5.2.3 behaved similarly to mAb 4.1.3, while monoclonal antibodies 4.8 and 12.3.9 were similar to mAb 5.12.14. Thus, mAb 4.1.3 and mAb 5.2.3 bind to a different epitope(s) than the epitope recognized by monoclonal antibodies 12.3.9, 4.8 and 5.12.14.

Immunodot blot analysis was performed to assess antibody reactivity to IL-8 immobilized on nitrocellulose paper. All seven antibodies recognized IL-8 immobilized on paper, whereas a control mouse IgG antibody did not.

The ability of these monoclonal antibodies to capture soluble ¹²⁵I-IL-8 was assessed by a

radioimmune precipitation test (RIP). Briefly, tracer ^{125}I -IL-8 (4×10^4 cpm) was incubated with various dilutions of the monoclonal anti-IL-8 antibodies in 0.2 ml of PBS containing 0.5% BSA and 0.05% Tween 20 (assay buffer) for 1 hr at room temperature. One hundred microliters of a predetermined concentration of goat anti-mouse Ig antisera (Pel-Freez, Rogers, AR) were added and the mixture was incubated at room temperature for 1 hr. Immune complexes were precipitated by the addition of 0.5 ml of 6% polyethylene glycol (M.W. 8000) kept at 4°C. After centrifugation at $2,000 \times g$ for 20 min at 4°C, the supernatant was removed by aspiration and the radioactivity remaining in the pellet was counted in a gamma counter. Percent specific binding was calculated as (precipitated cpm - background cpm) / (total cpm - background cpm). Monoclonal antibodies 4.1.3, 5.2.3, 4.8, 5.12.14 and 12.3.9 captured ^{125}I -IL-8 very efficiently, while antibodies 9.2.4 and 8.9.1 were not able to capture soluble ^{125}I -IL-8 in the RIP even though they could bind to IL-8 coated onto ELISA plates (Table I).

The dissociation constants of these monoclonal antibodies were determined using a competitive binding RIP assay. Briefly, competitive inhibition of the binding each antibody to ^{125}I -IL-8 (20,000-40,000 cpm per assay) by various amounts of unlabeled IL-8 was determined by the RIP described above. The dissociation constant (affinity) of each mAb was determined by using Scatchard plot analysis (Munson, *et al.*, Anal. Biochem. 107:220 (1980)) as provided in the VersaTerm-PRO computer program (Synergy Software, Reading, PA). The K_d 's of these monoclonal antibodies (with the exception of 9.2.4 and 8.9.1) were in the range from 2×10^{-8} to 3×10^{-10} M. Monoclonal antibody 5.12.14 with a K_d of 3×10^{-10} M showed the highest affinity among all the monoclonal antibodies tested (Table 3).

Table 3. Characterization of Anti-IL-8 Monoclonal Antibodies

Antibody	%Specific Binding to IL-8	K_d (M)	Isotype	pI
4.1.3	58	2×10^{-9}	IgG ₁	4.3-6.1
5.2.3	34	2×10^{-8}	IgG ₁	5.2-5.6
9.2.4	1	-	IgG ₁	7.0-7.5
8.9.1	2	-	IgG ₁	6.8-7.6

Antibody	%Specific Binding to IL-8	$K_d(M)$	Isotype	pI
4.8	62	3×10^{-8}	IgG _{2a}	6.1-7.1
5.12.14	98	3×10^{-10}	IgG _{2a}	6.2-7.4
12.3.9	86	2×10^{-9}	IgG _{2a}	6.5-7.1

To assess the ability of these monoclonal antibodies to neutralize IL-8 activity, the amount of ¹²⁵I-IL-8 bound to human neutrophils in the presence of various amounts of culture supernatants and purified monoclonal antibodies was measured. Neutrophils were prepared by using Mono-Poly Resolving Medium (M-PRM) (Flow Lab. Inc., McLean, VA). Briefly fresh, heparinized human blood was loaded onto M-PRM at a ratio of blood to medium, 3.5:3.0, and centrifuged at 300 x g for 30 min at room temperature. Neutrophils enriched at the middle layer were collected and washed once in PBS. Such a preparation routinely contained greater than 95% neutrophils according to the Wright's Giemsa staining. The receptor binding assay was done as follows. 50 µl of ¹²⁵I-IL-8 (5 ng/ml) was incubated with 50 µl of unlabeled IL-8 (100 µg/ml) or monoclonal antibodies in PBS containing 0.1% BSA for 30 min at room temperature. The mixture was then incubated with 100 µl of neutrophils (10⁷ cells/ml) for 15 min at 37°C. The ¹²⁵I-IL-8 bound was separated from the unbound material by loading mixtures onto 0.4 ml of PBS containing 20% sucrose and 0.1% BSA and by centrifugation at 300 x g for 15 min. The supernatant was removed by aspiration and the radioactivity associated with the pellet was counted in a gamma counter.

Monoclonal antibodies 4.1.3, 5.2.3, 4.8, 5.12.14, and 12.3.9 inhibited greater than 85% of the binding of IL-8 to human neutrophils at a 1:25 molar ratio of IL-8 to mAb. On the other hand, monoclonal antibodies 9.2.4 and 8.9.1 appeared to enhance the binding of IL-8 to its receptors on human neutrophils. Since a control mouse IgG also enhanced the binding of IL-8 on neutrophils, the enhancement of IL-8 binding to its receptors by mAb 9.2.4 and 8.9.1 appears to be nonspecific. Thus, monoclonal antibodies, 4.1.3, 5.1.3, 4.8, 5.12.14, and 12.3.9 are potential neutralizing monoclonal antibodies while monoclonal antibodies 8.9.1 and 9.2.4 are non-neutralizing monoclonal antibodies.

The ability of the anti-IL-8 antibodies to block neutrophil chemotaxis induced by IL-8 was tested as follows. Neutrophil chemotaxis induced by IL-8 was determined using a Boyden chamber method

(Larsen, *et al.* Science 243:1464 (1989)). One hundred μ l of human neutrophils (10^6 cells/ml) resuspended in RPMI containing 0.1% BSA were placed in the upper chamber and 29 μ l of the IL-8 (20 nM) with or without monoclonal antibodies were placed in the lower chamber. Cells were incubated for 1 hr at 37°C. Neutrophils migrated into the lower chamber were stained with Wright's Giemsa stain and counted under the microscope (100x magnification). Approximately 10 different fields per experimental group were examined. Neutralizing monoclonal antibodies 5.12.14 and 4.1.3 blocked almost 70% of the neutrophil chemotactic activity of IL-8 at 1:10 ratio of IL-8 to mAb.

The isoelectric focusing (IEF) pattern of each mAb was determined by applying purified antibodies on an IEF polyacrylamide gel (pH 3-9, Pharmacia) using the Fast gel system (Pharmacia, Piscataway, NJ). The IEF gel was pretreated with pharmalyte containing 1% Triton X100 (Sigma, St. Louis, MO) for 10 min before loading the samples. The IEF pattern was visualized by silver staining according to the instructions from the manufacturer. All of the monoclonal antibodies had different IEF patterns, confirming that they originated from different clones. The pI values for the antibodies are listed in Table 3.

All these monoclonal antibodies bound equally well to both (ala-IL-8)77 and (ser-IL-8)72 forms of IL-8. Because IL-8 has greater than 30% sequence homology with certain other members of the platelet factor 4 (PF4) family of inflammatory cytokines such as β -TG (Van Damme *et al.*, Eur. J. Biochem. 181:337(1989); Tanaka *et al.*, FEB 236(2):467 (1988)) and PF4 (Deuel *et al.*, Proc. Natl. Acad. Sci. U.S.A. 74:2256 (1977)), they were tested for possible cross reactivity to β -TG and PF4, as well as to another neutrophil activating factor, C5a. No detectable binding to any of these proteins was observed, with the exception of mAb 4.1.3, which had a slight cross reactivity to β -TG.

One of the antibodies, mAb 5.12.14, was further studied to determine whether it could block the IL-8 mediated release of elastase by neutrophils. Briefly, human neutrophils were resuspended in Hanks balanced salt solution (Gibco, Grand Island, NY) containing 1.0% BSA, Fraction V (Sigma, St. Louis, MO), 2 mg/ml alpha-D-glucose (Sigma), 4.2 mM sodium bicarbonate (Sigma) and 0.01 M HEPES, pH 7.1 (JRH Bioscience, Lenexa, KS). A stock of cytochalasin B (Sigma) was prepared (5 mg/ml in dimethylsulfoxide (Sigma) and stored at 2-8°C. Cytochalasin B was added to the neutrophil preparation to produce a final concentration of 5 μ g/ml, and incubated for 15 min at 37°C. Human IL-8 was incubated with mAb 5.12.14 (20 μ l), or a negative control antibody, in 1 ml polypropylene tubes (DBM Scientific, San Fernando, CA) for 30 min at 37°C. The final assay concentrations of IL-8 were 50 and 500 nM. The monoclonal antibodies were diluted to produce the following ratios (IL-8:Mab): 1:50, 1:10, 1:2, 1:1, and 1:0.25. Cytochalasin B-treated neutrophils were added (100 μ l/tube) and incubated for 2 hours at 25°C. The tubes were centrifuged (210 X g, 2-8°C) for 10 min, and supernatants were transferred to 96 well tissue culture plates (30 μ l/well). Elastase substrate stock, 10 mM methoxysuccinyl-alanyl-alanyl-propyl-valyl-p-nitroanilide (Calbiochem, La Jolla, CA) in DMSO was prepared and stored at 2-8°C. Elastase substrate solution (1.2 mM substrate, 1.2 M NaCl (Mallinckrodt, Paris, Kentucky), 0.12 M HEPES pH 7.2 in distilled water) was added (170 μ l/well) to the supernatants and incubated for 0.5 to 2 hours at 37°C (until control

O.D. of 1.0 was reached). Absorbance was measured at 405 nm (SLT 340 ATTC plate reader, SLT Lab Instruments, Austria).

The results are shown in Figure 1. At a 1:1 ratio of IL-8 to mAb 5.12.14, the antibody was able to effectively block the release of elastase from neutrophils.

5 The hybridoma producing antibody 5.12.14 was deposited on February 15, 1993 with the American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD, U.S.A. (ATCC) and assigned ATCC Accession No. HB 11553.

B. GENERATION AND CHARACTERIZATION OF MONOCLONAL ANTIBODIES AGAINST RABBIT IL-8

10 Antibodies against rabbit IL-8 were generated in essentially the same process as anti-human IL-8 antibodies using rabbit IL-8 as immunogen (kindly provided by C. Broaddus; see also Yoshimura *et al.* J. Immunol. 146:3483 (1991)). The antibody was characterized as described above for binding to other cytokines coated onto ELISA plates; no measurable binding was found to MGSA, fMLP, C5a, b-TG, TNF, PF4, or IL-1.

15 The hybridoma producing antibody 6G4.2.5 was deposited on September 28, 1994, with the American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD, U.S.A. (ATCC) and assigned ATCC Accession No. HB 11722.

Recombinant human-murine chimeric Fabs for 5.12.14 and 6G4.2.5 were constructed as described below. A chimeric 6G4.2.5 Fab is compared with a chimeric 5.12.14 Fab in detail below.

20 1. INHIBITION OF IL-8 BINDING TO HUMAN NEUTROPHILS BY 5.12.14-FAB AND 6G4.2.5-FAB

The ability of the two chimeric Fabs, 5.12.14-Fab and 6G4.2.5-Fab, to efficiently bind IL-8 and prevent IL-8 from binding to IL-8 receptors on human neutrophils was determined by performing a competition binding assay which allows the calculation of the IC_{50} - concentration required to achieve 50% inhibition of IL-8 binding.

25 Human neutrophils (5×10^5), were incubated for 1 hour at 4°C with 0.5nM ^{125}I -IL-8 in the presence of various concentrations (0 to 300 nM) of 5.12.14-Fab, 6G4.2.5-Fab, an isotype control (4D5-Fab) or unlabeled IL-8. After the incubation, the unbound ^{125}I -IL-8 was removed by centrifugation through a solution of 20% sucrose and 0.1% bovine serum albumin in phosphate buffered saline and the amount of ^{125}I -IL-8 bound to the cells was determined by counting the cell pellets in a gamma counter. Figure 2 demonstrates the inhibition of ^{125}I -IL-8 binding to neutrophils by unlabeled IL-8. Figure 3 demonstrates that a negative isotype matched Fab does not inhibit the binding of ^{125}I -IL-8 to human neutrophils. Both the anti-IL-8 Fabs, 5.12.14 Fab (Figure 4) and 6G4.2.5 Fab (Figure 5) were able to inhibit the binding of ^{125}I -IL-8 to human neutrophils with an average IC_{50} of 1.6 nM and 7.5 nM, respectively.

2. INHIBITION OF IL-8-MEDIATED NEUTROPHIL CHEMOTAXIS BY 5.12.14-FAB AND 6G4.2.5-FAB

Human neutrophils were isolated, counted and resuspended at 5×10^6 cells/ml in Hank's balanced salt solution (abbreviated HBSS; without calcium and magnesium) with 0.1% bovine serum albumin. The neutrophils were labeled by adding calcein AM (Molecular Probe, Eugene, OR) at a final concentration of 2.0 μ M. Following a 30 minute incubation at 37°C, cells were washed twice with HBSS-BSA and resuspended at 5×10^6 cells/ml.

Chemotaxis experiments were carried out in a Neuro Probe (Cabin John, MD) 96-well chamber, model MBB96. Experimental samples (buffer only control, IL-8 alone or IL-8 + Fabs) were loaded in a Polyfiltronics 96-well View plate (Neuro Probe Inc.) placed in the lower chamber. 100 μ l of the calcein AM-labeled neutrophils were added to the upper chambers and allowed to migrate through a 5 micrometer porosity PVP free polycarbonate framed filter (Neuro Probe Inc.) toward the bottom chamber sample. The chemotaxis apparatus was then incubated for 40 to 60 minutes at 37°C with 5% CO₂. At the end of the incubation, neutrophils remaining in the upper chamber were aspirated and upper chambers were washed three times with PBS. Then the polycarbonate filter was removed, non-migrating cells were wiped off with a squeegee wetted with PBS, and the filter was air dried for 15 minutes.

The relative number of neutrophils migrating through the filter (Neutrophil migration index) was determined by measuring fluorescence intensity of the filter and the fluorescence intensity of the contents of the lower chamber and adding the two values together. Fluorescence intensity was measured with a CytoFluor 2300 fluorescent plate reader (Millipore Corp. Bedford, MA) configured to read a Corning 96-well plate using the 485-20 nm excitation filter and a 530-25 emission filter, with the sensitivity set at 3.

The results are shown in Figures 6 and 7. Figure 6 demonstrates the inhibition of human IL-8 mediated neutrophil chemotaxis by chimeric 6G4.2.5 and 5.12.14 Fabs. Figure 7 demonstrates the relative abilities of chimeric 6G4.2.5 and 5.12.14 Fabs to inhibit rabbit IL-8 mediated neutrophil chemotaxis.

3. INHIBITION OF IL-8-MEDIATED NEUTROPHIL ELASTASE RELEASE BY VARIOUS CONCENTRATIONS OF 6G4.2.5 AND 5.12.14 FABS

Blood was drawn from healthy male donors into heparinized syringes. Neutrophils were isolated by dextran sedimentation, centrifugation over Lymphocyte Separation Medium (Organon Teknika, Durham, NC), and hypotonic lysis of contaminating red blood cells as described by Berman *et al.* (*J. Cell Biochem.* 52:183 (1993)). The final neutrophil pellet was suspended at a concentration of 1×10^7 cells/ml in assay buffer, which consisted of Hanks Balanced Salt Solution (GIBCO, Grand Island, NY) supplemented with 1.0% BSA (fraction V, Sigma, St. Louis, MO), 2 mg/ml glucose, 4.2 mM sodium bicarbonate, and 0.01 M HEPES, pH 7.2. The neutrophils were stored at 4°C for not longer than 1 hr.

IL-8 (10 μ l) was mixed with anti-IL-8 Fab, an isotype control Fab, or buffer (20 μ l) in 1 ml polypropylene tubes and incubated in a 37°C water bath for 30 min. IL-8 was used at final concentrations ranging from 0.01 to 1000 nM in dose response studies (Figure 8) and at a final concentration of 100 nM in

the experiments addressing the effects of the Fabs on elastase release (Figures 9 and 10). Fab concentrations ranged from approximately 20 nM to 300 nM, resulting in Fab:IL-8 molar ratios of 0.2:1 to 3:1. Cytochalasin B (Sigma) was added to the neutrophil suspension at a concentration of 5 µg/ml (using a 5 mg/ml stock solution made up in DMSO), and the cells were incubated for 15 min in a 37°C water bath.

5 Cytochalasin B-treated neutrophils (100 µl) were then added to the IL-8/Fab mixtures. After a 3 hr incubation at room temperature, the neutrophils were pelleted by centrifugation (200 x g for 5 min), and aliquots of the cell-free supernatants were transferred to 96 well plates (30 µl/well). The elastase substrate, methoxysuccinyl-alanyl-alanyl-prolyl-valyl-p-nitroanilide (Calbiochem, La Jolla, CA), was prepared as a 10 mM stock solution in DMSO and stored at 4°C. Elastase substrate working solution was prepared just prior

10 to use (1.2 mM elastase substrate, 1.2 M NaCl, 0.12 M HEPES, pH 7.2), and 170 µl was added to each sample-containing well. The plates were placed in a 37°C tissue culture incubator for 30 min or until an optical density reading for the positive controls reached at least 1.0. Absorbance was measured at 405 nm using an SLT 340 plate reader (SLT Lab Instruments, Austria).

Figure 9 demonstrates the ability of the chimeric anti-IL-8 Fabs to inhibit elastase release from human neutrophils stimulated by human IL-8; Figure 10 demonstrates the relative abilities of the chimeric

15 anti-IL-8 Fabs to inhibit elastase release from human neutrophils stimulated by rabbit IL-8.

C. MOLECULAR CLONING OF THE VARIABLE LIGHT AND HEAVY REGIONS OF THE MURINE 5.12.14 (ANTI-IL-8) MONOCLONAL ANTIBODY

Total RNA was isolated from 1×10^8 cells (hybridoma cell line ATCC HB-11722) using the

20 procedure described by Chomczynski and Sacchi (Anal. Biochem. 162:156 (1987)). First strand cDNA was synthesized by specifically priming the mRNA with synthetic DNA oligonucleotides designed to hybridize with regions of the murine RNA encoding the constant region of the kappa light chain or the IgG2a heavy chain (the DNA sequence of these regions are published in Sequences of Proteins of Immunological Interest, Kabat, E. A. *et al.* (1991) NIH Publication 91-3242, V 1-3.). Three primers (SEQ ID NOS: 1-6) were

25 designed for each of the light and heavy chains to increase the chances of primer hybridization and efficiency of first strand cDNA synthesis (Figure 13). Amplification of the first strand cDNA to double-stranded (ds) DNA was accomplished using two sets of synthetic DNA oligonucleotide primers: one forward primer (SEQ ID NOS: 7-9) and one reverse primer (SEQ ID NO: 10) for the light chain variable region amplification (Figure 14) and one forward primer (SEQ ID NOS: 11-14) and one reverse primer

30 (SEQ ID NOS: 15-18) for the heavy chain variable region amplification (Figure 15). The N-terminal sequence of the first eight amino acids of either the light or heavy chains of 5.12.14 was used to generate a putative murine DNA sequence corresponding to this region. (A total of 29 amino acids was sequenced from the N-terminus of both the light chain and heavy chain variable regions using the Edman degradation protein sequencing technique.) This information was used to design the forward amplification primers

35 which were made degenerate in the third position for some codons to increase the chances of primer hybridization to the natural murine DNA codons and also included the unique restriction site, MluI, for both the light chain variable region forward primer and the heavy chain variable region forward primer to

facilitate ligation to the 3' end of the STII element in the cloning vector. The reverse amplification primers were designed to anneal with the murine DNA sequence corresponding to a portion of the constant region of the light or heavy chains near the variable/constant junction. The light chain variable region reverse primer contained a unique BstBI restriction site and the heavy chain variable region reverse primer contained a unique Apal restriction site for ligation to the 5' end of either the human IgG1 constant light or IgG1 constant heavy regions in the vectors, pB13.1 (light chain) and pB14 (heavy chain). The polymerase chain reaction using these primer sets yielded DNA fragments of approximately 400 bp. The cDNA encoding the 5.12.14 light chain variable region was cloned into the vector pB13.1, to form pA51214VL and the 5.12.14 heavy chain variable region was cloned into the vector, pB14, to form pA51214VH. The cDNA inserts were characterized by DNA sequencing and are presented in the DNA sequence (SEQ ID NO: 19) and amino acid sequence (SEQ ID NO: 20) of Figure 16 (murine light chain variable region) and in the DNA sequence (SEQ ID NO: 21) and amino acid (SEQ ID NO: 22) of Figure 17 (murine heavy chain variable region).

D. CONSTRUCTION OF A 5.12.14 FAB VECTOR

In the initial construct, pA51214VL, the amino acids between the end of the 5.12.14 murine light chain variable sequence and the unique cloning site, BstBI, in the human IgG1 constant light sequence were of murine origin corresponding to the first 13 amino acids of the murine IgG1 constant region (Figure 16). Therefore, this plasmid contained a superfluous portion of the murine constant region separating the 5.12.14 murine light chain variable region and the human light chain IgG1 constant region. This intervening sequence would alter the amino acid sequence of the chimera and most likely produce an incorrectly folded Fab. This problem was addressed by immediately truncating the cDNA clone after A109 and re-positioning the BstBI site to the variable/constant junction by the polymerase chain reaction. Figure 18 shows the amplification primers used to make these modifications. The forward primer, VL.front (SEQ ID NO: 23), was designed to match the last five amino acids of the STII signal sequence, including the MluI cloning site, and the first 4 amino acids of the 5.12.14 murine light chain variable sequence. The sequence was altered from the original cDNA in the third position of the first two codons D1 (T to C) and I2 (C to T) to create a unique EcoRV cloning site which was used for later constructions. The reverse primer, VL.rear (SEQ ID NO: 24), was designed to match the first three amino acids of the human IgG1 constant light sequence and the last seven amino acids of the 5.12.14 light chain variable sequence which included a unique BstBI cloning site. In the process of adding the BstBI site, the nucleotide sequence encoding several amino acids were altered: L106 (TTG to CTT), K107 (AAA to CGA) resulting in a conservative amino acid substitution to arginine, and R108 (CGG to AGA). The PCR product encoding the modified 5.12.14 light chain variable sequence was then subcloned into pB13.1 in a two-part ligation. The MluI-BstBI digested 5.12.14 PCR product encoding the light chain variable region was ligated into MluI-BstBI digested vector to form the plasmid, pA51214VL'. The modified cDNA was characterized by DNA sequencing. The coding sequence for the 5.12.14 light chain is shown in Figure 19.

Likewise, the DNA sequence between the end of the heavy chain variable region and the unique

cloning site, Apal, in the human IgG1 heavy chain constant domain of pA51214VH was reconstructed to change the amino acids in this area from murine to human. This was done by the polymerase chain reaction. Amplification of the murine 5.12.14 heavy chain variable sequence was accomplished using the primers shown in Figure 18. The forward PCR primer (SEQ ID NO: 25) was designed to match nucleotides 867-887 in pA51214VH upstream of the STII signal sequence and the putative cDNA sequence encoding the heavy chain variable region and included the unique cloning site SpeI. The reverse PCR primer (SEQ ID NO: 26) was designed to match the last four amino acids of the 5.12.14 heavy chain variable sequence and the first six amino acids corresponding to the human IgG1 heavy constant sequence which also included the unique cloning site, Apal. The PCR product encoding the modified 5.12.14 heavy chain variable sequence was then subcloned to the expression plasmid, pMHM24.2.28 in a two-part ligation. The vector was digested with SpeI-Apal and the SpeI-Apal digested 5.12.14 PCR product encoding the heavy chain variable region was ligated into it to form the plasmid, pA51214VH'. The modified cDNA was characterized by DNA sequencing. The coding sequence for the 5.12.14 heavy chain is shown in the DNA sequence (SEQ ID NO: 29) and amino acid sequence (SEQ ID NO: 30) of Figures 20A-20B.

The first expression plasmid, pantilL-8.1, encoding the chimeric Fab of 5.12.14 was made by digesting pA51214VH' with EcoRV and Bpu1102I to replace the EcoRV-Bpu1102I fragment with a EcoRV-Bpu1102I fragment encoding the murine 5.12.14 light chain variable region of pA51214VL'. The resultant plasmid thus contained the murine-human variable/constant regions of both the light and heavy chains of 5.12.14.

Preliminary analysis of Fab expression using pantilL-8.1 showed that the light and heavy chains were produced intracellularly but very little was being secreted into the periplasmic space of *E. coli*. To correct this problem, a second expression plasmid was constructed.

The second expression plasmid, pantilL-8.2, was constructed using the plasmid, pmy187, as the vector. Plasmid pantilL-8.2 was made by digesting pmy187 with MluI and SphI and the MluI (partial)-SphI fragment encoding the murine 5.12.14 murine-human chimeric Fab of pantilL-8.1 was ligated into it. The resultant plasmid thus contained the murine-human variable/constant regions of both the light and heavy chains of 5.12.14.

The plasmid pantilL-8.2 was deposited on February 10, 1995 with the American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD, U.S.A. (ATCC) and assigned ATCC Accession No. ATCC 97056.

E. MOLECULAR CLONING OF THE VARIABLE LIGHT AND HEAVY REGIONS OF THE MURINE 6G4.2.5 MONOCLONAL ANTIBODY

Total RNA was isolated from 1×10^8 cells (hybridoma cell line 6G4.2.5) using the procedure described by Chomczynski and Sacchi (*Anal. Biochem.* 162:156 (1987)). First strand cDNA was synthesized by specifically priming the mRNA with synthetic DNA oligonucleotides designed to hybridize with regions of the murine RNA encoding the constant region of the kappa light chain or the IgG2a heavy chain (the DNA sequence of these regions are published in Sequences of Proteins of Immunological Interest,

Kabat *et al.* (1991) NIH Publication 91-3242, V 1-3). Three primers (SEQ ID NOS: 31-36) were designed for each the light and heavy chains to increase the chances of primer hybridization and efficiency of first strand cDNA synthesis (Figure 21). Amplification of the first strand cDNA to double-stranded (ds) DNA was accomplished using two sets of synthetic DNA oligonucleotide primers: one forward primer (SEQ ID NOS: 37-39) and one reverse primer (SEQ ID NO: 40) for the light chain variable region amplification (Figure 22) and one forward primer (SEQ ID NOS: 41-42) and one reverse primer (SEQ ID NOS: 43-46) for the heavy chain variable region amplification (Figure 23). The N-terminal sequence of the first eight amino acids of either the light or heavy chains of 6G4.2.5 was used to generate a putative murine DNA sequence corresponding to this region. (A total of 29 amino acids were sequenced from the N-terminus of both the light chain and heavy chain variable regions using the Edman degradation protein sequencing technique.) This information was used to design the forward amplification primers which were made degenerate in the third position for some codons to increase the chances of primer hybridization to the natural murine DNA codons and also included the unique restriction site, NsiI, for the light chain variable region forward primer and the unique restriction site, MluI, for the heavy chain variable region forward primer to facilitate ligation to the 3' end of the STII element in the vector, pchimFab. The reverse amplification primers were designed to anneal with the murine DNA sequence corresponding to a portion of the constant region of the light or heavy chains near the variable/constant junction. The light chain variable region reverse primer contained a unique MunI restriction site and the heavy chain variable region reverse primer contained a unique ApaI restriction site for ligation to the 5' end of either the human IgG1 constant light or IgG1 constant heavy regions in the vector, pchimFab. The polymerase chain reaction using these primer sets yielded DNA fragments of approximately 400 bp and were cloned individually into the vector, pchimFab, to form p6G425VL and p6G425VH. The cDNA inserts were characterized by DNA sequencing and are presented in the DNA sequence (SEQ ID NO: 47) and amino acid sequence (SEQ ID NO: 48) of Figure 24 (murine light chain variable region) and the DNA sequence (SEQ ID NO: 49) and amino acid sequence (SEQ ID NO: 50) of Figure 25 (murine heavy chain variable region).

F. CONSTRUCTION OF A 6G4.2.5 CHIMERIC FAB VECTOR

In the initial construct, p6G425VL, the amino acids between the end of the 6G4.2.5 murine light chain variable sequence and the unique cloning site, MunI, in the human IgG1 constant light sequence were of murine origin. These amino acids must match the human IgG1 amino acid sequence to allow proper folding of the chimeric Fab. Two murine amino acids, D115 and S121, differed dramatically from the amino acids found in the loops of the β -strands of the human IgG1 constant domain and were converted to the proper human amino acid residues, V115 and F121, by site-directed mutagenesis using the primers (SEQ ID NOS: 51-54) shown in Figure 26. These specific mutations were confirmed by DNA sequencing and the modified plasmid named p6G425VL'. The coding sequence is shown in the DNA sequence (SEQ ID NO: 55) and amino acid sequence (SEQ ID NO: 56) of Figures 27A-27B.

Likewise, the DNA sequence between the end of the heavy chain variable region and the unique cloning site, ApaI, in the human IgG1 heavy chain constant domain of p6G425VH was reconstructed to

change the amino acids in this area from murine to human. This process was facilitated by the discovery of a BstEII site near the end of the heavy chain variable region. This site and the Apal site were used for the addition of a synthetic piece of DNA encoding the corresponding IgG human amino acid sequence. The synthetic oligo-nucleotides shown in Figure 26 were designed as complements of one another to allow the formation of a 27 bp piece of ds DNA. The construction was performed as a three-part ligation because the plasmid, p6G425VH, contained an additional BstEII site within the vector sequence. A 5309 bp fragment of p6G425VH digested with MluI-ApaI was ligated to a 388 bp fragment carrying the 6G4.2.5 heavy chain variable region and a 27 bp synthetic DNA fragment encoding the first six amino acids of the human IgG1 constant region to form the plasmid, p6G425VH'. The insertion of the synthetic piece of DNA was confirmed by DNA sequencing. The coding sequence is shown in the DNA sequence (SEQ ID NO: 57) and amino acid sequence (SEQ ID NO: 58) of Figures 28A-28B.

The expression plasmid, p6G425chim2, encoding the chimeric Fab of 6G4.2.5 was made by digesting p6G425chimVL' with MluI and ApaI to remove the STII-murine HPC4 heavy chain variable region and replacing it with the MluI-ApaI fragment encoding the STII-murine 6G4.2.5 heavy chain variable region of p6G425chimVH'. The resultant plasmid thus contained the murine-human variable/constant regions of both the light and heavy chains of 6G4.2.5.

The plasmid p6G425chim2 was deposited on February 10, 1995 with the American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD, U.S.A. (ATCC) and assigned ATCC Accession No. 97055.

20 G. CONSTRUCTION OF HUMANIZED VERSIONS OF ANTI-IL-8 ANTIBODY 6G4.2.5

The murine cDNA sequence information obtained from the hybridoma cell line, 6G4.2.5, was used to construct recombinant humanized variants of the murine anti-IL-8 antibody. The first humanized variant, F(ab)-1, was made by grafting synthetic DNA oligonucleotide primers encoding the murine CDRs of the heavy and light chains onto a phagemid vector, pEMX1 (Werther *et al.*, J. Immunol., 157: 4986-4995 (1996)), which contains a human 6-subgroup I light chain and a human IgG1 subgroup III heavy chain (Fig. 29). Amino acids comprising the framework of the antibody that were potentially important for maintaining the conformations necessary for high affinity binding to IL-8 by the complementarity-determining regions (CDR) were identified by comparing molecular models of the murine and humanized 6G4.2.5 (F(ab)-1) variable domains using methods described by Carter *et al.*, PNAS 89:4285 (1992) and Eigenbrot, *et al.*, J. Mol. Biol. 229:969 (1993). Additional humanized framework variants (F(ab) 2-9) were constructed from the information obtained from these models and are presented in Table 4 below. In these variants, the site-directed mutagenesis methods of Kunkel, Proc. Natl. Acad. Sci USA, 82:488 (1985) were utilized to exchange specific human framework residues with their corresponding 6G4.2.5 murine counterparts. Subsequently, the entire coding sequence of each variant was confirmed by DNA sequencing. Expression and purification of each F(ab) variant was performed as previously described by Werther *et al.*, *supra*, with the exception that hen egg white lysozyme was omitted from the purification protocol. The variant antibodies were analyzed by SDS-PAGE, electrospray mass spectroscopy and amino acid analysis.

Table 4 - Humanized 6G425 Variants

IC50^c

+-----+

Variant	Version	Template	Changes ^a	Purpose ^b	Mean	S.D.	N
F(ab)-1	version 1		CDR Swap		63.0	12.3	4
F(ab)-2	version 2	F(ab)-1	PheH67 <i>Ala</i>	packaging w/ CDR H2	106.0	17.0	2
F(ab)-3	version 3	F(ab)-1	ArgH71 <i>Val</i>	packaging w/ CDRs H1, H2	79.8	42.2	4
F(ab)-4	version 6	F(ab)-1	IleH69 <i>Leu</i>	packaging w/ CDR H2	44.7	9.0	3
F(ab)-5	version 7	F(ab)-1	LeuH78 <i>Ala</i>	packaging w/ CDRs H1, H2	52.7	31.0	9
F(ab)-6	version 8	F(ab)-1	IleH69 <i>Leu</i> LeuH78 <i>Ala</i>	combine F(ab)- 4 and -5	34.6	6.7	7
F(ab)-7	version 16	F(ab)-6	LeuH80 <i>Val</i>	packaging w/ CDR H1	38.4	9.1	2
F(ab)-8	version 19	F(ab)-6	ArgH38 <i>Lys</i>	packaging w/ CDR H2	14.0	5.7	2
F(ab)-9	version 11	F(ab)-6	GluH6 <i>Gln</i>	packaging w/ CDR H3	19.0	5.1	7
Chimeric ^d F(ab)					11.4	7.0	13
rhu4D5 ^c F(ab)					>200μM		5

- 5 a Amino acid changes made relative to the template used. Murine residues are in bold italics and residue numbering is according to Kabat *et al.*
- b Purpose for making changes based upon interactions observed in molecular models of the humanized and murine variable domains.
- 10 c nM concentration of variant necessary to inhibit binding of iodinated IL-8 to human neutrophils in the competitive binding assay.
- d Chimeric F(ab) is a (F(ab) which carries the murine heavy and light chain variable domains fused to the human light chain κI constant domain and the human heavy chain subgroup III constant domain I respectively.
- 15

- e. rhu4D5F(ab) is of the same isotype as the humanized 6G425 F(ab)s and is a humanized anti-HER2 F(ab) and therefore should not bind to IL8.

The first humanized variant, F(ab)-1, was an unaltered CDR swap in which all the murine CDR amino acids defined by both x-ray crystallography and sequence hypervariability were transferred to the human framework. When the purified F(ab) was tested for its ability to inhibit ¹²⁵I-IL-8 binding to human neutrophils according to the methods described in Section (B)(1) above, a 5.5 fold reduction in binding affinity was evident as shown in Table 4 above. Subsequent versions of F(ab)-1 were engineered to fashion the 3-dimensional structure of the CDR loops into a more favorable conformation for binding IL-8. The relative affinities of the F(ab) variants determined from competition binding experiments using human neutrophils as described in Section (B)(1) above are presented in Table 4 above. A slight decrease in IL-8 binding (<2 fold) was observed for F(ab)-2-3 while only slight increases in IL-8 binding were noted for F(ab)3-5. Variant F(ab)-6 had the highest increase in affinity for IL-8 (approximately 2 fold), exhibiting an IL-8 binding affinity of 34.6nM compared to the F(ab)-1 IL-8 binding affinity of 63nM. The substitutions of murine Leu for Ile at H69 and murine Ala for Leu at H78 are predicted to influence the packing of CDRs H1 and H2. Further framework substitutions using the F(ab)-6 variant as template were made to bring the binding affinity closer to that of the chimeric F(ab). *In-vitro* binding experiments revealed no change in affinity for F(ab)-7 (38.4nM) but a significant improvement in affinity for F(ab)-8/9 of 14nM and 19 nM, respectively. By analysis of a 3-D computer-generated model of the anti-IL-8 antibody, it was hypothesized that the substitution of murine Lys for Arg at H38 in F(ab)-8 influences CDR-H2 while a change at H6 of murine Gln for Glu in F(ab)-9 affects CDR-H3. Examination of the human antibody sequences with respect to amino acid variability revealed that the frequency of Arg at residue H38 is >99% whereas residue H6 is either Gln ~20% or Glu ~80% (Kabat *et. al.*, Sequences of Proteins of Immunological Interest 5th Ed. (1991)). Therefore, to reduce the likelihood of causing an immune response to the antibody, F(ab)-9 was chosen over F(ab)-8 for further affinity maturation studies. Variant F(ab)-9 was also tested for its ability to inhibit IL-8-mediated chemotaxis (Fig. 30). This antibody was able to block neutrophil migration induced by wild-type human IL-8, human monomeric IL-8 and Rhesus IL-8 with IC₅₀s of approximately 12nM, 15nM, and 22nM, respectively, in IL-8 mediated neutrophil chemotaxis inhibition assays performed as described in Section (B)(2) above. The amino acid sequence for variant F(ab)-8 is provided in Fig. 31c. The F(ab)-8 was found to block human and rhesus IL-8-mediated chemotaxis with IC₅₀s of 12nM and 10nM, respectively, in IL-8 mediated neutrophil chemotaxis inhibition assays performed as described in Section (B)(2) above.

H. CONSTRUCTION OF AN ANTI-IL-8-GENE III FUSION PROTEIN FOR PHAGE DISPLAY AND ALANINE SCANNING MUTAGENESIS

An expression plasmid, pPh6G4.V11, encoding a fusion protein (heavy chain of the humanized 6G4.2.5 version 11 antibody and the M13 phage gene-III coat protein) and the light chain of the humanized 6G4.2.5 version 11 antibody was assembled to produce a monovalent display of the anti-IL-8 antibody on

phage particles. The construct was made by digesting the plasmid, pFPHX, with EcoRV and Apal to remove the existing irrelevant antibody coding sequence and replacing it with a 1305bp EcoRV-Apal fragment from the plasmid, p6G4.V11, encoding the humanized 6G4.2.5 version 11 anti-IL-8 antibody. The translated sequence of the humanized 6G4.2.5 version 11 heavy chain (SEQ ID NO: 66), peptide linker and gene III coat protein (SEQ ID NO: 67) is shown in Fig. 31A. The pFPHX plasmid is a derivative of pHGHam-3 which contains an in-frame amber codon (TAG) between the human growth hormone and gene-III DNA coding sequences. When transformed into an amber suppressor strain of *E. coli*, the codon (TAG) is read as Glutamate producing a growth hormone (hGH)-gene III fusion protein. Likewise, in a normal strain of *E. coli*, the codon (TAG) is read as a stop preventing translational read-through into the gene-III sequence and thus allowing the production of soluble hGH. The pGHam-3 plasmid is described in Methods: A Companion to Methods in Enzymology, 3:205 (1991). The final product, pPh6G4.V11, was used as the template for the alanine scanning mutagenesis of the CDRs and for the construction of randomized CDR libraries of the humanized 6G4.V11 antibody.

1. ALANINE SCANNING MUTAGENESIS OF HUMANIZED ANTIBODY 6G4.2.5 VERSION 11

The solvent exposed amino acid residues in the CDRs of the humanized anti-IL-8 6G4.2.5 version 11 antibody (h6G4V11) were identified by analysis of a 3-D computer-generated model of the anti-IL-8 antibody. In order to determine which solvent exposed amino acids in the CDRs affect binding to interleukin-8, each of the solvent exposed amino acids was individually changed to alanine, creating a panel of mutant antibodies wherein each mutant contained an alanine substitution at a single solvent exposed residue. The alanine scanning mutagenesis was performed as described by Leong *et. al.*, J. Biol. Chem., 269: 19343 (1994)).

The IC₅₀'s (relative affinities) of h6G4V11 wt and mutated antibodies were established using a Competition Phage ELISA Assay described by Cunningham *et. al.*, (EMBO J. 13:2508 (1994)) and Lee *et. al.*, (Science 270:1657 (1995)). The assay measures the ability of each antibody to bind IL-8 coated onto a 96-well plate in the presence of various concentrations of free IL-8 (0.2 to 1uM) in solution. The first step of the assay requires that the concentrations of the phage carrying the wild type and mutated antibodies be normalized, allowing a comparison of the relative affinities of each antibody. The normalization was accomplished by titrating the phage on the IL-8 coated plates and establishing their EC₅₀. Sulfhydryl coated 96-well binding plates (Corning-Costar; Wilmington, MA) were incubated with a 0.1mg/ml solution of K64C IL-8 (Lysine 64 is substituted with Cysteine to allow the formation of a disulfide bond between the free thiol group of K64C IL-8 and the sulfhydryl coated plate, which results in the positioning of the IL-8 receptor binding domains towards the solution interface) in phosphate buffered saline (PBS) pH 6.5 containing 1mM EDTA for 1 hour at 25EC followed by three washes with PBS and a final incubation with a solution of PBS containing 1.75mg/ml of L-cysteine-HCl and 0.1M NaHCO₃ to block any free reactive sulfhydryl groups on the plate. The plates were washed once more and stored covered at 4EC with 200ul of PBS/well. Phage displaying either the reference antibody, h6G4V11, or the mutant h6G4V11 antibodies were grown and harvested by PEG precipitation. The phage were resuspended in 500ul 10mM Tris-HCl pH

7.5, 1mM EDTA and 100mM NaCl and held at 4EC for no longer than 3 hours. An aliquot of each phage was diluted 4-fold in PBS containing 0.05% Tween-20 (BioRad, Richmond, Ca.) and 0.5% BSA RIA grade (Sigma, St. Louis, Mo.) (PBB) and added to IL-8 coated plates blocked for at least 2 hours at 25EC with 50mg/ml skim milk powder in 25mM Carbonate Buffer pH 9.6. The phage were next serially diluted in 3 fold steps down the plate from well A through H. The plates were incubated for 1 hour at 25EC followed by nine quick washes with PBS containing 0.05% Tween-20 (PBST). The plates were then incubated with a 1:3200 dilution of rabbit anti-phage antibody and a 1:1600 dilution of secondary goat-anti-rabbit Fc HRP-conjugated antibody for 15 minutes at 25EC followed by nine quick washes with PBST. The plates were developed with 80ul/well of 1mg/ml OPD (Sigma, St. Louis, Mo) in Citrate Phosphate buffer pH 5.0 containing 0.015% H₂O₂ for 4 minutes at 25EC and the reaction stopped with the addition of 40ul of 4.5M H₂SO₄. The plates were analyzed at wavelength 8492 in a SLT model 340ATTC plate reader (SLT Lab Instruments). The individual EC₅₀s were determined by analyzing the data using the program Kaleidagraph (Synergy Software, Reading, Pa.) and a 4-parameter fit equation. The phage held at 4EC were then immediately diluted in PBB to achieve a final concentration corresponding to their respective EC₅₀ or target OD₄₉₂ for the competition segment of the experiment, and dispensed into a 96 well plate containing 4-fold serial dilutions of soluble IL-8 ranging from 1uM in well A and ending with 0.2uM in well H. Using a 12-channel pipet, 100ul of the phage/IL-8 mixture was transferred to an IL-8 coated 96-well plate and executed as described above. Each sample was done in triplicate - 3 columns/sample.

Table 5 - Relative Affinities (IC₅₀) for Alanine-scan Anti-IL-8 6G4V11 CDR Mutants

CDR	Amino Acid Residue	Avg IC ₅₀ (nM)	Std Dev
V11	Reference	11.5	6.4
CDR-L1	S26	6.3	2.9
	Q27	10.2	2.4
	S28	14.2	5.2
	V30	29.1	12.3
	H31	580.3	243.0
	I33	64.2	14.6
	N35	3.3	0.7
	T36	138.0	nd
CDR-L2	Y37	NDB	nd
	K55	24.2	14.9
	V56	15.5	3.8
	S57	12.4	4.0
	N58	17.6	3.7
	R59	nd	nd
CDR-L3	S96	10.8	4.4
	T97	70.6	55.2

CDR	Amino Acid Residue	Avg IC50 (nM)	Std Dev
	H98	8.0	1.2
	V99	19.6	1.9
CDR-H1	S28	8.6	3.1
	S30	nd	nd
	S31	7.8	2.5
	H32	13.3	5.8
	Y53	48.2	15.8
CDR-H2	Y50	35.6	13.0
	D52	13.3	7.5
	S53	6.0	3.4
	N54	96.0	5.8
	E56	15.8	4.5
	T57	8.4	1.6
	T58	11.3	1.8
	Y59	9.1	3.7
	Q61	12.6	6.4
	K64	18.5	12.1
CDR-H3	D96	NDB	nd
	Y97	NDB	nd
	R98	36.6	15.3
	Y99	199.5	nd
	N100	278.3	169.4
	D102	159.2	44
	W103	NDB	nd
	F104	NDB	nd
	F105	209.4	72.3
	D106	25.3	21.7

Each sample performed in triplicate/experiment.

NDB = No Detectable Binding /nd = value not determined*

Residue numbering is according to Kabat et al.

- 5 The results of the alanine-scan are summarized in Table 5 above. The alanine substitutions in of many of the mutant antibodies had little or no adverse effects (<3 fold) on the binding affinity for IL-8. Mutants that were found to exhibit no detectable binding of IL-8 (NDB) presumably contained disruptions in the conformational structure of the antibody conferred by crucial structural or buried amino acids in the CDR. Based on the results of the scan, CDR-H3 (heavy chain, 3rd CDR) was identified as the dominant
- 10 binding epitope for binding IL-8. Alanine substitutions in this CDR resulted in a 3 to >26 fold decrease in binding affinity. The amino acids, Y597, Y599 and D602 are of particular interest because it was determined from the computer generated model of the anti-IL-8 antibody that these residues are solvent exposed and that these residues might participate in hydrogen bonding or charge interactions with IL-8 or other amino acids of the antibody that influence either binding to IL-8 or the conformation of the CDR-H3

loop structure. (See the model depicted in Fig. 32). Unexpected increases in binding affinity (1.8 > 2.7 fold) were not d for S528 and S531 of CDR-H1 and S553 of CDR-H2.

Surprisingly, a significant increase in binding affinity was observed in the alanine mutant N35A located in CDR-L1 (light chain, 1st CDR). A 3-6 fold increase in affinity was observed compared to the wild-type h6G4V11 antibody. This augmentation of IL-8 binding could be the result of the close proximity of N35A to CDR-H3. The alanine substitution may have imparted a slight change in the conformation of CDR-L1 which alters the packing interaction of neighboring amino acid residues on CDR-H3, thereby tweaking the loop of CDR-H3 into a conformation that facilitates more appropriate contacts with IL-8. Similarly, N35A may also influence the orientation of amino acids in CDR-L1 or its interaction directly with IL-8. Unexpected increases in affinity (~2 fold) were also observed for S26 of CDR-L1 and H98 of CDR-L3.

J. CHARACTERIZATION OF HUMANIZED ANTI-IL-8 ANTIBODY 6G4V11N35A

Soluble 6G4V11N35A Fab antibody was made by transforming an amber non-suppressor strain of *E. coli*, 34B8, with pPh6G4.V11 and growing the culture in low phosphate medium for 24 hours. The periplasmic fraction was collected and passed over a Hi-Trap Protein-G column (Pharmacia, Piscataway, NJ.) followed by a desalting and concentration step. The protein was analyzed by SDS-PAGE, mass spectrometry and amino acid analysis. The protein had the correct size and amino acid composition (Fig. 35). The 6G4V11N35A Fab was tested for its ability to inhibit ¹²⁵I-IL-8 binding to human neutrophils and to inhibit IL-8 mediated neutrophil chemotaxis as described in Section (B)(1) and (B)(2) above. As shown in Fig. 33, hybridoma-derived intact murine antibody (6G4 murine mAB), recombinant 6G4 murine-human chimera Fab, recombinant humanized Fab versions 1 and 11, and 6G4V11N35A Fab were found to inhibit ¹²⁵I-IL-8 binding to human neutrophils with an average IC₅₀ of 5nM, 8nM, 40nM, 10nM and 3nM, respectively. The 6G4V11N35A Fab had at least a 2-fold higher affinity than the 6G4.2.5 chimera Fab and a 3-fold higher affinity than 6G4V11. As shown in Fig. 34, the 6G4V11N35A Fab was found to inhibit IL-8 mediated neutrophil chemotaxis induced by both wild type and monomeric human IL-8, and by two different animal species of IL-8, namely, rabbit and rhesus. The irrelevant isotype control Fab (4D5) did not inhibit neutrophil migration. The average IC₅₀ values were 3nM (wt IL-8), 1 nM (monomeric IL-8), 5nM (Rabbit IL-8), and 10nM (Rhesus IL-8).

K. CONSTRUCTION OF A 6G4V11N35A F(ab')₂ LEUCINE ZIPPER

Production of a F(ab')₂ version of the humanized anti-IL-8 6G4V11N35A Fab was accomplished by constructing a fusion protein with the yeast GCN4 leucine zipper. The expression plasmid p6G4V11N35A.F(ab')₂ was made by digesting the plasmid p6G425chim2.fab2 with the restriction enzymes *bsa*I and *apa*I to remove the DNA sequence encoding the 6G4.2.5 murine-human chimeric Fab and replacing it with a 2620bp *bsa*I-*apa*I fragment from pPh6G4.V11N35A. The plasmid p6G425chim2.fab2 is a derivative of pS1130 which encodes a fusion protein (the GCN4-leucine zipper fused to the heavy chain of

anti-CD18) and the light chain of anti-CD18 antibody. The expression plasmid p6G4V11N35A.F(ab')₂ was deposited on February 20, 1996 with the American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD, U.S.A. (ATCC) and assigned ATCC Accession No. 97890. A pepsin cleavage site in the hinge region of the antibody facilitates the removal of the leucine zipper leaving the two immunoglobulin monomers joined by the cysteines that generate the interchain disulfide bonds. The DNA and protein sequence of the h6G4V11N35A.F(ab')₂ are depicted in Figs. 35-37.

An expression host cell was obtained by transforming E. coli strain 49D6 with p6G4V11N35A.F(ab')₂ essentially as described in Section (II)(3)(C) above. The transformed host E. coli 49D6 (p6G4V11N35A.F(ab')₂) was deposited on February 20, 1997 at the ATCC and assigned ATCC Accession No. 98332. Transformed host cells were grown in culture, and the 6G4V11N35A F(ab')₂ product was harvested from the host cell periplasmic space essentially as described in Section (II)(3)(F) above.

L. CHARACTERIZATION OF THE HUMANIZED 6G4V11N35A F(ab')₂ LEUCINE ZIPPER

The 6G4V11N35A Fab and F(ab')₂ were tested for their ability to inhibit ¹²⁵I-IL-8 binding to neutrophils according to the procedures described in Section (B)(1) above. The displacement curves from a representative binding experiment performed in duplicate is depicted in Fig. 38. Scatchard analysis of this data shows that 6G4V11N35A F(ab')₂ inhibited ¹²⁵I-IL-8 binding to human neutrophils with an average IC₅₀ of 0.7 nM (+/- 0.2). This is at least a 7 fold increase in affinity compared to the hybridoma-derived intact murine antibody (average IC₅₀ of 5 nM) and at least a 2.8 fold increase in affinity over the Fab version (average IC₅₀ of 2 nM).

The 6G4V11N35A F(ab')₂ was also tested for its ability to inhibit IL-8 mediated neutrophil chemotaxis according to the procedures described in Section (B)(2) above. The results of a representative chemotaxis experiment performed in quadruplicate are depicted in Fig. 39. As shown in Fig. 39, the 6G4V11N35A F(ab')₂ inhibited human IL-8 mediated neutrophil chemotaxis. The 6G4V11N35A F(ab')₂ exhibited an average IC₅₀ value of 1.5nM versus 2.7nM for the 6G4V11N35A Fab, which represents an approximately 2 fold improvement in the antibody's ability to neutralize the effects of IL-8. The irrelevant isotype control Fab (4D5) did not inhibit neutrophil migration. Furthermore, the 6G4V11N35A F(ab')₂ antibody retained its ability to inhibit IL-8 mediated neutrophil chemotaxis by monomeric IL-8 and by two different animal species of IL-8, namely rabbit and rhesus, in neutrophil chemotaxis experiments conducted as described above. An individual experiment is shown in Fig. 40. The average IC₅₀ values were 1nM (monomeric IL-8), 4nM (Rabbit IL-8), and 2.0nM (Rhesus IL-8).

M. RANDOM MUTAGENESIS OF LIGHT CHAIN AMINO ACID (N35A) IN CDR-L1 OF HUMANIZED ANTIBODY 6G4V11

A 3-fold improvement in the IC₅₀ for inhibiting ¹²⁵I-IL-8 binding to human neutrophils was observed when alanine was substituted for asparagine at position 35 in CDR-L1 (light chain) of the humanized 6G4V11 mAb as described in Section (I) above. This result might be attributed to an improvement in the contact between the antigen-antibody binding interfaces as a consequence of the replacement of a less bulky nonpolar side chain (R-group) that may have altered the conformation of CDR-L1 or neighboring CDR-H3 (heavy chain) to become more accessible for antigen docking. The acceptance of alanine at position 35 of CDR-L1 suggested that this position contributed to improved affinity and that an assessment of the re-modeling of CDR loops / antigen-binding region(s) by other amino acids at this location was warranted. Selection of an affinity matured version of the humanized 6G4.V11 mAB (Kunkel, T. A., Proc. Natl. Acad. Sci. USA, 82:488 (1995)) was accomplished by randomly mutagenizing position 35 of CDR-L1 and constructing an antibody-phage library. The codon for Asparagine (N) at position 35 of CDR-L1, was targeted for randomization to any of the 20 known amino acids.

Initially, a stop template, pPH6G4.V11-stop, was made to eliminate contaminating wild-type N35 sequence from the library. This was accomplished by performing site-directed mutagenesis (Muta-Gene Kit, Biorad, Richmond, CA) of pPH6G4V11 (described in Section (H) above) to replace the codon (AAC) for N35 with a stop codon (TAA) using the primer SL.97.2 (SEQ ID NO:)(Figure 42). The incorporation of the stop codon was confirmed by DNA sequencing. Subsequently, uracil containing single-stranded DNA derived from *E. coli* CJ236 transformed with the stop template was used to generate an antibody-phage library following the method described by Lowman (Methods in Molecular Biology, 87 Chapter 25: 1-15 (1997)). The variants generated from this library were predicted to produce a collection of antibodies containing one of the 20 known amino acids at position N35 in CDR-L1. The amino acid substitutions were accomplished by site-directed mutagenesis using the degenerate oligonucleotide primer (SL.97.3) with the sequence NNS (N = A/G/T/C ; S = G/C;) (SEQ ID NO:)(Figure 42). This codon usage should allow for the expression of any of the 20 amino acids - including the amber stop codon (TAG). The collection of antibody-phage variants was transfected into *E. coli* strain XL-1 blue (Stratagene, San Diego, CA) by electroporation and grown at 37°C overnight to amplify the library. Selection of tight binding humanized 6G4V11 Fab's were accomplished by panning the library on IL-8 coated 96-well plates as described in Section (I) above. Prior to panning, the number of phage/library was normalized to 1.1×10^{13} phage/ml (which produces a maximum OD₂₇₀ reading = 1 OD unit) and IL-8 coated plates were incubated with blocking solution (25mN Carbonate buffer containing 50mg/ml skim milk) for 2 hours before the addition of phage (each sort used eight IL-8 coated wells/library). After the blocking and washing steps, every sort began with the addition of 100ul of antibody-phage (titered at 1.1×10^{13} phage/ml) to each of eight IL-8 coated wells followed by an 1 hour incubation at 25°C. The non-specifically bound antibody-phage were removed by 10 quick washes with PBS-0.05% Tween 20 (PBS-

Tween). For sort #1, a low stringency wash (100ul PBS-Tween/well for 10 minutes at 25°C) was employed to capture the small proportion of tight binding antibody-phage bound to the immobilized IL-8. The antibody-phage variants specifically bound to IL-8 were eluted with 100ul/well of 200mM Glycine pH 2.0 for 5 minutes at 25°C. The eluted antibody-phage variants from the 8 wells were then pooled and
5 neutralized with 1M Tris-HCl pH 8.0 (1/3 the elution volume). The phage were titered and propagated as described in Section (I) above. The stringency of the washes were successively increased with each round of panning depending upon the percent recovery of phage at the end of a sort. The wash conditions were as follows: sort #2 (4 x 15 minute intervals; total time = 60 minutes) and sort #3 (either #3a: 8 x 15 minute intervals or #3b: 12 x 10 minute intervals; total time = 120 minutes). The total number of phage recovered
10 was progressively reduced after each sort suggesting that non- or weak- binders were being selected against. The recovery of the negative control (the antibody-phage stop variant) was constant throughout the panning (approximately 0.0001 to 0.00001 percent).

Eighteen random variants from sort #3 were analyzed by DNA sequencing to look for an amino acid consensus at position 35 of CDR-L1. The data presented in Figure 43A showed that Glycine
15 occupied position 35 in 33% of the variants sequenced. However, after correcting for the number of NNS codon combinations/amino acid, the frequency of Glycine was reduced to 16.6%. Glutamic Acid was represented with the highest frequency (22%) followed by Aspartic Acid and Glycine (16.6%). The frequencies of recovery of the wild-type Asparagine and substituted Alanine were only 5.6%. Interestingly, the high frequency of Glycine may suggest that a much wider range of conformations might be allowed for
20 the loop of CDR-L1 which may be attributed to the reduction in steric hindrance of bond angle (ϕ - ψ) pairing as a result of the single hydrogen atom as the side chain. Conversely, Glutamic Acid at position 35 might restrict the flexibility of the loop by imposing less freedom of rotation imposed by the more rigid and bulky charged polar side chain.

Soluble Fab's of the affinity matured variants (N35G, N35D, N35E and N35A) were made as
25 described in Section (J) above for evaluating their ability to block IL-8 binding. As shown in Figure 43B, variants N35A, N35D, N35E and N35G were found to inhibit ¹²⁵I-IL-8 binding to human neutrophils with an approximate IC₅₀ of 0.2nM, 0.9nM, 0.1nM and 3.0nM, respectively. All of the affinity matured variants showed an improvement in binding IL-8 ranging from 3 - 100 fold compared to the humanized 6G4V11 mAb. The affinity-matured variant, 6G4V11N35E, was 2-fold more potent in blocking IL-8
30 binding to human neutrophils than the alanine-scan variant, 6G4V11N35A.

Equilibrium and kinetic measurements of variants 6G4V11N35A and 6G4V11N35E were determined using KinEXATM automated immunoassay system (Sapidyne Instruments Inc., Idaho City, ID) as described by Blake *et al.*, J. Biol. Chem. 271: 27677 (1996). The procedure for preparing the antigen-coated particles was modified as follows: 1 ml of activated agarose beads (Reacti-Gel 6X; Pierce,
35 Rockford, IL) were coated with antigen in 50mM Carbonate buffer pH 9.6 containing 20ug/ml of human IL-8 and incubated with gentle agitation on a rocker overnight at 25°C. The IL-8 coated beads were then

washed twice with 1M Tris-HCl pH 7.5 to inactivate any unreactive groups on the beads and blocked with Superblock (Pierce, Rockford, IL) for 1 hour at 25C to reduce non-specific binding. The beads were resuspended in assay buffer (0.1% bovine serum albumin in PBS) to a final volume of 30 ml. A 550ul aliquot of the IL-8 coated bead suspension was used each time to pack a fresh 4mm high column in the KinEXA observation cell. The amount of unbound antibody from the antibody-antigen mixtures captured by the IL-8-coated beads in both the equilibrium and kinetic experiments was quantified using a fluorescently labeled secondary antibody. Murine 6G4.2.5 was detected with a R-PE AffiniPure F(ab')₂ goat anti-mouse IgG, Fc fragment specific 2° antibody (Jackson Immuno Research Laboratories, West Grove, PA) and humanized affinity matured N35A (Fab and F(ab')₂) and N35E Fab were detected with a R-PE AffiniPure F(ab')₂ donkey anti-human IgG (H+L) 2° antibody (Jackson ImmunoResearch Laboratories, West Grove, PA); both at a 1:1000 dilution.

Equilibrium measurements were determined by incubating a constant amount of anti-IL-8 antibody (0.005ug/ml) with various concentrations of human IL-8 (0, 0.009, 0.019, 0.039, 0.078, 0.156, 0.312, 0.625, 1.25, 2.5nM). The antibody-antigen mixture was incubated for 2 hours at 25°C to allow the molecules to reach equilibrium. Subsequently, each sample was passed over a naive IL-8 coated bead pack in the KinEXA observation cell at a flow rate of 0.5ml/minute for a total of 9 minutes/sample. The equilibrium constant (K_d) was calculated using the software provided by Sapidyne Instruments Inc.

Rates of association (k_a) and dissociation (k_d) were determined by incubating together a constant amount of antibody and antigen, and measuring the amount of uncomplexed anti-IL-8 bound to the IL-8 coated beads over time. The concentration of antibody used in the kinetic experiments was identical to that used in the equilibrium experiment described above. Generally, the amount of human IL-8 used was the concentration derived from the binding curves of the equilibrium experiment that resulted in 70% inhibition of anti-IL-8 binding to the IL-8 coated beads. Measurements were made every 15 minutes to collect approximately nine data points. The k_a was calculated using the software provided by Sapidyne Instruments, Inc. The off rate was determined using the equation: $k_d = K_d/k_a$.

Figure 44 shows the equilibrium constants (K_d) for the affinity matured variants 6G4V11N35E and 6G4V11N35A Fab's were approximately 54pM and 114pM, respectively. The improvement in affinity of 6G4V11N35E Fab for IL-8 can be attributed to a 2-fold faster rate of association (K_{on}) of 4.7×10^6 for 6G4V11N35E Fab versus 2.0×10^6 for 6G4V11N35A F(ab')₂. (The K_d of the 6G4V11N35A F(ab')₂ and 6G4V11N35A Fab are similar.) The dissociation rates (K_{off}) were not significantly different. Molecular modeling suggests that substitution of Asparagine with Glutamic Acid might either affect the antibody's interaction with IL-8 directly or indirectly by neutralizing the charge of neighboring residues R98 (CDR-H3) or K50 (CDR-L2) in the CDR's to facilitate contact with IL-8. Another effect might be the formation of a more stable loop conformation for CDR-L1 that could have facilitated more appropriate contacts of other CDR-L1 loop residues with IL-8. The DNA (SEQ-ID-NO:) and amino acid (SEQ-ID-NO:)

sequences of p6G4V11N35E.Fab showing the Asparagine to Glutamic Acid substitution in the light chain are present in Figure 45.

N. CHARACTERIZATION OF HUMANIZED ANTI-IL-8 VARIANT 6G4V11N35E Fab

The affinity matured Fab variant, 6G4V11N35E, was tested for its ability to inhibit IL-8 mediated neutrophil chemotaxis as described in Section (B)(2) above. The reusable 96-well chemotaxis chamber described in Section (B)(2) was replaced with endotoxin-free disposable chemotaxis chambers containing 5-micron PVP-free polycarbonate filters (ChemoTx101-5, Neuro Probe, Inc. Cabin John, MD). As illustrated in Figure 46, variant N35E effectively blocks IL-8 mediated neutrophil chemotaxis induced by a 2nM stimulus of either rabbit or human IL-8. In fact, the level of inhibition at antibody concentrations between 3.7nM - 33nM was not significantly different from the buffer control indicating variant N35E could completely inhibit this response. The IC_{50} 's for both rabbit and human IL-8 were approximately 2.8nM and 1.2nM, respectively. The irrelevant isotype control Fab (4D5) did not inhibit neutrophil migration indicating the results observed for the affinity matured variant, N35E, is IL-8 specific.

O. CONSTRUCTION OF HUMANIZED 6G4V11N35E F(ab')₂ LEUCINE ZIPPER

A F(ab')₂ expression plasmid for 6G4V11N35E was constructed using methods similar to those described in Section (K) above. The expression plasmid, p6G4V11N35E.F(ab')₂, was made by digesting the plasmid p6G4V11N35A.F(ab')₂ (described in Section (K) above) with the restriction enzymes Apal and NdeI to isolate a 2805 bp fragment encoding the heavy chain constant domain -GCN4 leucine zipper and ligating it to a 3758 bp Apal-NdeI fragment of the pH6G4V11N35E phage display clone (encoding 6G4V11N35E Fab) obtained as described in Section (M) above. The integrity of the entire coding sequence was confirmed by DNA sequencing.

P. CONSTRUCTION OF THE FULL LENGTH HUMANIZED 6G4V11N35A IgG EXPRESSION PLASMID

The full length IgG₁ version of the humanized anti-IL8 variant 6G4V11N35A was made using a dicistronic DHFR-Intron expression vector (Lucas et al., Nucleic Acids Res., 24: 1774-1779 (1996)) which contained the full length recombinant murine-human chimera of the 6G4.2.5 anti-IL8 mAb. The expression plasmid encoding the humanized variant 6G4V11N35A was assembled as follows. First an intermediate plasmid (pSL-3) was made to shuttle the sequence encoding the variable heavy chain of humanized anti-IL-8 variant 6G4V11N35A to pRK56G4chim.2Vh - which contains the variable heavy region of the chimeric 6G4.5 anti-IL8 antibody. The vector pRK56G4chim.Vh was digested with PvuII and Apal to remove the heavy chain variable region of the chimeric antibody and religated with an 80bp PvuII - XhoI synthetic oligonucleotide (encoding Leu4 to Phe29 of 6G4V11N35A) (Fig. 47) and a 291bp XhoI - Apal fragment from p6G4V11N35A.7 carrying the remainder of the variable heavy chain sequence of 6G4V11N35A to create pSL-3. This intermediate plasmid was used in conjunction with 2 other plasmids, p6G4V11N35A.F(ab')₂ and p6G425chim2.choSD, to create the mammalian expression plasmid,

p6G4V11N35AchoSD.9 (identified as p6G425V11N35A.choSD in a deposit made on December 16, 1997 with the ATCC and assigned ATCC Accession No. 209552). This expression construct was assembled in a 4-part ligation using the following DNA fragments: a 5,203bp ClaI - BlnI fragment encoding the regulatory elements of the mammalian expression plasmid (p6G425 chim2.choSD), a 451bp ClaI - ApaI fragment containing the heavy chain variable region of the humanized 6G4V11N35A antibody (pSL-3), a 1,921bp ApaI - EcoRV fragment carrying the heavy chain constant region of 6G4V11N35A (p6G425chim2.choSD) and a 554bp EcoRV - BlnI fragment encoding the light chain variable and constant regions of 6G4V11N35A (p6G4V11N35A.F(ab')₂). The DNA sequence (SEQ ID NO:) of clone p6G4V11N35A.choSD.9 was confirmed by DNA sequencing and is presented in Figure 48.

10 Q. CONSTRUCTION OF THE FULL LENGTH HUMANIZED 6G4V11N35E IgG EXPRESSION PLASMID

A mammalian expression vector for the humanized 6G4V11N35E was made by swapping the light chain variable region of 6G4V11N35A with 6G4V11N35E as follows: a 7,566bp EcoRV - BlnI fragment (void of the 554bp fragment encoding the light chain variable region of 6G4V11N35A) from p6G4V11N35A.choSD.9 was ligated to a 554bp EcoRV - BlnI fragment (encoding the light chain variable region of 6G4V11N35E) from pPH6G4V11N35E.7. The mutation at position N35 of the light chain of p6G4V11N35E.choSD.10 was confirmed by DNA sequencing.

R. STABLE CHO CELL LINES FOR VARIANTS N35A AND N35E

For stable expression of the final humanized IgG1 variants (6G4V11N35A and 6G4V11N35E), Chinese hamster ovary (CHO) DP-12 cells were transfected with the above-described dicistronic vectors (p6G4V11N35A.choSD.9 and p6G4V11N35E.choSD.10, respectively) designed to coexpress both heavy and light chains (Lucas et al., *Nucleic Acid Res.* 24:1774-79 (1996)). Plasmids were introduced into CHO DP12 cells via lipofection and selected for growth in GHT-free medium (Chisholm, V. High efficiency gene transfer in mammalian cells. In: Glover, DM, Hames, BD. *DNA Cloning 4. Mammalian systems.* Oxford Univ. Press, Oxford pp 1-41 (1996)). Approximately 20 unamplified clones were randomly chosen and reseeded into 96 well plates. Relative specific productivity of each colony was monitored using an ELISA to quantitate the full length human IgG accumulated in each well after 3 days and a fluorescent dye, Calcein AM, as a surrogate marker of viable cell number per well. Based on these data, several unamplified clones were chosen for further amplification in the presence of increasing concentrations of methotrexate. Individual clones surviving at 10, 50, and 100 nM methotrexate were chosen and transferred to 96 well plates for productivity screening. One clone for each antibody (clone#1933 aIL8.92 NB 28605/12 for 6G4V11N35A; clone#1934 aIL8.42 NB 28605/14 for 6G4V11N35E), which reproducibly exhibited high specific productivity, was expanded in T-flasks and used to inoculate a spinner culture. After several passages, the suspension-adapted cells were used to inoculate production cultures in GHT-containing, serum-free media supplemented with various hormones and protein hydrolysates. Harvested cell culture fluid containing recombinant humanized anti-IL8 was purified using protein A-Sepharose CL-4B. The purity after this step was approximately 99%. Subsequent purification to homogeneity was carried out

using an ion exchange chromatography step. Production titer of the humanized 6G4V11N35E IgG1 antibody after the first round of amplification and 6G4V11N35A IgG1 after the second round of amplification were 250mg/L and 150mg/L, respectively.

S. CHARACTERIZATION OF THE HUMANIZED 6G4V11N35A/E IgG VARIANTS

5 The humanized full length IgG variants of 6G4.2.5 were tested for their ability to inhibit 125 I-IL-8 binding and to neutralize activation of human neutrophils; the procedures are described in Sections (B)(1) and (B)(2) above. As shown in Figure 49, the full length IgG1 forms of variants 6G4V11N35A and 6G4V11N35E equally inhibited 125 I-IL-8 binding to human neutrophils with approximate IC_{50} 's of 0.3nM and 0.5nM, respectively. This represents a 15 - 25 fold improvement in blocking binding of IL-8 compared to the full length murine mAb (IC_{50} = 7.5nM). Similarly, the two anti-IL-8 variants showed equivalent neutralizing capabilities with respect to inhibiting IL-8 mediated human neutrophil chemotaxis (Figures 50A-50B). The IC_{50} 's of 6G4V11N35A IgG1 and 6G4V11N35E IgG1 for human IL-8 were 4.0nM and 6.0nM, respectively, and for rabbit IL-8 were 4.0nM and 2.0nM, respectively. The irrelevant isotype control Fab (4D5) did not inhibit neutrophil migration.

15 The affinity for IL-8 of these variants relative to the murine 6G4.2.5 mAb was determined using KinExA as described in Section (M). Figure 51 shows the equilibrium constant (Kd) for the full length affinity matured variants 6G4V11N35E IgG1 and 6G4V11N35A IgG1 were approximately 49pM and 88pM, respectively. The Kd for 6G4V11N35A IgG1 was determined directly from the kinetic experiment. As reported with their respective Fabs, this improvement in affinity might be attributed to an approximate 20 2-fold increase in the on-rate of 6G4V11N35E IgG1 (k_a = 3.0×10^6) compared to that of 6G4V11N35A IgG1 (k_a = 8.7×10^5). In addition, these results were confirmed by a competition radio-immune assay using iodinated human IL-8. 50pM of 6G4V11N35A IgG1 or 6G4V11N35E IgG1 was incubated for 2 hours at 25°C with 30-50pM of 125 I-IL-8 and varying concentrations (0 to 100nM) of unlabeled IL-8. The antibody-antigen mixture was then incubated for 1 hour at 4°C with 10ul of a 70% slurry of Protein-A beads (pre-blocked with 0.1% BSA). The beads were briefly spun in a microcentrifuge and the supernatant discarded to remove the unbound 125 I-IL-8. The amount of 125 I-IL-8 specifically bound to the anti-IL-8 25 antibodies was determined by counting the protein-A pellets in a gamma counter. The approximate Kd values were similar to those determined by KinEXA. The average Kd for 6G4V11N35A IgG1 and 6G4V11N35E IgG1 were 54pM (18 -90pM) and 19pM (5-34pM), respectively (Figure 52).

30 T. CONSTRUCTION OF HUMANIZED 6G4V11N35A/E Fab's FOR MODIFICATION BY POLYETHYLENE GLYCOL

A Fab' expression vector for 6G4V11N35A was constructed by digesting p6G4V11N35A.F(ab')₂ with the restriction enzymes ApaI and NdeI to remove the 2805 bp fragment encoding the human IgG₁

constant domain fused with the yeast GCN4 leucine zipper and replacing it with the 2683bp Apal-NdeI fragment from the plasmid pCDNA.18 described in Eigenbrot et al., *Proteins: Struct. Funct. Genet.*, **18**: 49-62 (1994). The pCDNA.18 Apal-NdeI fragment carries the coding sequence for the human constant IgG1 heavy domain, including the free cysteine in the hinge region that was used to attach the PEG molecule.

5 The 3758bp Apal-NdeI fragment (encodes the light chain and heavy variable domain of 6G4V11N35A) isolated from p6G4V11N35A.F(ab')₂ was ligated to the 2683bp Apal-NdeI fragment of pCDNA.18 to create p6G4V11N35A.PEG-1. The integrity of the entire coding sequence was confirmed by DNA sequencing. The nucleotide and translated amino acid sequences of heavy chain constant domain with the cysteine in the hinge are presented in Figure 53.

10 A Fab' expression plasmid for 6G4V11N35E was made similarly by digesting pPH6G4V11N35E (from Section (O) above) with the restriction enzymes Apal and NdeI to isolate the 3758bp Apal-NdeI DNA fragment carrying the intact light chain and heavy variable domain of 6G4V11N35E and ligating it to the 2683 bp Apal-NdeI DNA fragment from p6G4V11N35A.PEG-1 to create p6G4V11N35E.PEG-3. The integrity of the entire coding sequence was confirmed by DNA sequencing.

15 Anti-IL-8 6G4V11N35A Fab' variant was modified with 20 kD linear methoxy-PEG-maleimide, 30 kD linear methoxy-PEG-maleimide, 40 kD linear methoxy-PEG-maleimide, or 40 kD branched methoxy-PEG-maleimide as described below. All PEG's used were obtained commercially from Shearwater Polymers, Inc.

a. MATERIALS AND METHODS

Fab'-SH Purification

20 A Fab'-SH antibody fragment of the affinity matured antibody 6G4V11N35A was expressed in *E. coli* grown to high cell density in the fermentor as described by Carter *et al.*, *BioTechnology* **10**, 163-167 (1992). Preparation of Fab'-SH fragments was accomplished by protecting the Fab'-SH fragments with 4',4'-dithiodipyridine (PDS), partially purifying the protected Fab'-PDS fragments, deprotect the Fab'-PDS

25 with dithiothreitol (DTT) and finally isolate the free Fab'-SH by using gel permeation chromatography.

Protection of Fab'-SH with PDS

30 Fermentation paste samples were dissolved in 3 volumes of 20mM MES, 5mM EDTA, pH 6.0 containing 10.7mg of 4',4'-dithiodipyridine per gram fermentation paste, resulting in a suspension with a pH close to 6.0. The suspension was passed through a homogenizer followed by addition of 5% PEI (w/v), pH 6 to the homogenate to a final concentration of 0.25%. The mixture was then centrifuged to remove solids and the clear supernatant was conditioned to a conductivity of less than 3mS by the addition of cold water.

Partial purification of the Fab'-SH molecule using ion exchange chromatography

35 The conditioned supernatant was loaded onto an ABX (Baker) column equilibrated in 20 mM MES, pH 6.0. The column was washed with the equilibration buffer followed by elution of the Fab'-SH with a 15 column volume linear gradient from 20 mM MES, pH 6.0 to 20 mM MES, 350 mM sodium chloride. The column was monitored by absorbance at 280nm, and the eluate was collected in fractions.

Deprotection of the Fab'-SH antibody fragments with DTT

The pH of the ABX pool was adjusted to 4.0 by the addition of dilute HCl. The pH adjusted solution was then deprotected by adding DTT to a final concentration of 0.2mM. The solution was incubated for about 30 minutes and then applied to a gel filtration Sephadex G25 column, equilibrated with 15mM sodium phosphate, 25mM MES, pH 4.0. After elution, the pH of the pool was raised to pH 5.5 and immediately flash frozen at -70°C for storage or derivatized with PEG-MAL as described below.

Alternative Fab'-SH Purification

Alternatively Fab'-SH fragments can be purified using the following procedure. 100 g fermentation paste is thawed in the presence of 200 ml 50 mM acetic acid, pH 2.8, 2 mM EDTA, 1 mM PMSF. After mixing vigorously for 30 min at room temperature, the extract is incubated with 100 mg hen egg white lysozyme. DEAE fast flow resin (approximately 100 mL) is equilibrated with 10 mM MES, pH 5.5, 1 mM EDTA on a sintered glass funnel. The osmotic shock extract containing the Fab'-SH fragment is then filtered through the resin.

A protein G Sepharose column is equilibrated with 10 mM MES, pH 5.5, 1 mM EDTA and then loaded with the DEAE flow-through sample. The column is washed followed by three 4 column volume washes with 10 mM MES, pH 5.5, 1 mM EDTA. The Fab'-SH antibody fragment containing a free thiol is eluted from the column with 100 mM acetic acid, pH 2.8, 1 mM EDTA. After elution, the pH of the pool is raised to pH 5.5 and immediately flash frozen at -70°C for storage or derivatized with PEG-MAL as described below.

Preparation of Fab'-S-PEG

The free thiol content of the Fab'-SH preparation obtained as described above was determined by reaction with 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB) analysis according to the method of Creighton in Protein Structure: A Practical Approach, Creighton, T.E., ed, IRL Press (Oxford, UK: 1990), pp. 155-167. The concentration of free thiol was calculated from the increase on absorbance at 412 nm, using $\epsilon_{412} = 14,150 \text{ cm}^{-1} \text{ M}^{-1}$ for the thionitrobenzoate anion and a $M_r = 48,690$ and $\epsilon_{280} = 1.5$ for the Fab'-SH antibody. To the Fab'-SH protein G Sepharose pool, or the deprotected Fab'-SH gel permeation pool, 5 molar equivalents of PEG-MAL were added and the pH was immediately adjusted to pH 6.5 with 10% NaOH.

The Fab'-S-PEG was purified using a 2.5 x 20 cm cation exchange column (Poros 50-HS). The column was equilibrated with a buffer containing 20 mM MES, pH 5.5. The coupling reaction containing the PEGylated antibody fragment was diluted with deionized water to a conductivity of approximately 2.0 mS. The conditioned coupling reaction was then loaded onto the equilibrated Poros 50 HS column. Unreacted PEG-MAL was washed from the column with 2 column volumes of 20 mM MES, pH 5.5. The Fab'-S-PEG was eluted from the column using a linear gradient from 0 to 400 mM NaCl, in 20 mM MES pH 5.5, over 15 column volumes.

Alternatively a Bakerbond ABX column can be used to purify the Fab'-S-PEG molecule. The column is equilibrated with 20 mM MES, pH 6.0 (Buffer A). The coupling reaction is diluted with deionized water until the conductivity equaled that of the Buffer A (approximately 2.0 mS) and loaded onto the column. Unreacted PEG-MAL is washed from the column with 2 column volumes of 20 mM MES, pH 6.0. The Fab'-S-PEG is eluted from the column using a linear gradient from 0 to 100 mM (NH₄)₂SO₄, in 20 mM MES pH 6.0; over 15 column volumes.

Size Exclusion Chromatography

The hydrodynamic or effective size of each molecule was determined using a Pharmacia Superose-6 HR 10/30 column (10x300mm). The mobile phase was 200 mM NaCl, 50 mM sodium phosphate pH 6.0. Flow rate was at 0.5 ml/min and the column was kept at ambient temperature. Absorbance at 280 nm was monitored where PEG contributed little signal. Biorad MW standards containing cyanocobalamin, myoglobin, ovalbumin, IgG, Thyroglobulin monomer and dimer were used to generate a standard curve from which the effective size of the pegylated species was estimated.

b. RESULTS

Size Exclusion Chromatography

The effective size of each modified species was characterized using size exclusion chromatography. The results are shown in Fig. 60 below. The theoretical molecular weight of the anti-IL8 Fab fragments modified with PEG 5kD, 10kD, 20kD, 30kD, 40kD (linear), 40kD (branched) or 100,000kD is shown along with the apparent molecular weight of the PEGylated fragments obtained by HPLC size exclusion chromatography. When compared to the theoretical molecular weight of the Fab'-S-PEG fragments, the apparent molecular weight (calculated by size exclusion HPLC) increases dramatically by increasing the size of the PEG attached to the fragments. Attachment of a small molecular weight PEG, for example PEG 10,000D only increases the theoretical molecular weight of the PEGylated antibody fragment (59,700 D) by 3 fold to an apparent molecular weight of 180,000D. In contrast attachment of a larger molecular weight PEG for example 100,000D PEG to the antibody fragment increases the theoretical molecular weight of the PEGylated antibody fragment (158,700 D) by 12 fold to an apparent molecular weight of 2,000,000D.

SDS-PAGE

In Fig. 61, the upper panel shows the size of the anti-IL-8 Fab fragments modified with PEG of molecular weight 5kD (linear), 10kD (linear), 20kD (linear), 30kD (linear), 40kD (linear), 40kD (branched) or 100kD (linear) under reduced conditions. The unmodified Fab is shown in lane 2 from right to left. Both the heavy and light chains of the Fab had a molecular weight of approximately 30kD as determined by PAGE. Each PEGylated fragment sample produced two bands: (1) a first band (attributed to the light chain) exhibiting a molecular weight of 30kD; and (2) a second band (attributed to the heavy chain to which the PEG is attached specifically at the hinge SH) exhibiting increasing molecular weights of 40, 45, 70, 110, 125, 150 and 300kD. This result suggested that PEGylation was specifically restricted to the heavy chain of the Fab's whereas the light chain remained unmodified.

The lower panel is non-reduced PAGE showing the size of the anti-IL-8 Fab fragments modified with PEG of molecular weight 5kD (linear), 20kD (linear), 30kD (linear), 40kD (linear), 40kD (branched), or 100kD (linear). The PEGylated fragments exhibited molecular weights of approximately 70kD, 115kD, 120kD, 140kD, 200kD and 300kD.

- 5 The SDS PAGE gels confirm that all Fab'-S-PEG molecules were purified to homogeneity and that the molecules differed only with respect to the size of the PEG molecule attached to them.

U. AMINE SPECIFIC PEGYLATION OF ANTI-IL-8 F(ab')₂ FRAGMENTS

- Pegylated F(ab')₂ species were generated by using large MW or branched PEGs in order to achieve a large effective size with minimal protein modification which might affect activity. Modification involved N-hydroxysuccinamide chemistry which reacts with primary amines (lysines and the N-terminus). To decrease the probability of modifying the N-terminus, which is in close proximity to the CDR region, a reaction pH of 8, rather than the commonly used pH of 7, was employed. At pH 8.0, the amount of the reactive species (charged NH₃⁺) would be considerably more for the ε-NH₂ group of lysines (pK_a=10.3) than for the α-NH₂ group (pK_a of approximately 7) of the amino-terminus. For the linear PEGs, a methoxy-succinimidyl derivative of an NHS-PEG was used because of the significantly longer half-life in solution (17 minutes at 25°C at pH 8.0) compared to the NHS esters of PEGs (which have 5-7 minute half life under the above conditions). By using a PEG that is less prone to hydrolysis, a greater extent of modification is achieved with less PEG. Branched PEGs were used to induce a large increase in effective size of the antibody fragments.

20 a. MATERIALS

- All PEG reagents were purchased from Shearwater Polymers and stored at -70°C in a desiccator: branched N-hydroxysuccinamide-PEG (PEG2-NHS-40kDa) has a 20 kDa PEG on each of the two branches, methoxy-succinimidyl-propionic acid-PEG (M-SPA-20000) is a linear PEG molecule with 20 kDa PEG. Protein was recombinantly produced in *E. coli* and purified as a (Fab')₂ as described in Sections (K) and (O) above.

b. METHODS

- IEX method:** A J. T. Baker Wide-Pore Carboxy-sulfone (CSX), 5 micron, 7.75 x 100 mm HPLC column was used for fractionation of the different pegylated products, taking advantage of the difference in charge as the lysines are modified. The column was heated at 40°C. A gradient as shown in Table 7 below was used where Buffer A was 25 mM sodium Borate/25 mM sodium phosphate pH 6.0, and Buffer B was 1 M ammonium sulfate, and Buffer C was 50 mM sodium acetate pH 5.0.

Table 7

	Time (min)	%B	%C	flow mL/min
5	0	10	10	1.5
	20	18	7.5	1.5
	25	25	7.5	1.5
10	27	70	3.0	2.5
	29	70	3.0	2.5
	30	10	10	2.5
	33	10	10	2.5

15 **SEC-HPLC:** The hydrodynamic or effective size of each molecule was determined using a Pharmacia Superose-6 HR 10/30 column (10x300mm). The mobile phase was 200 mM NaCl, 50 mM sodium phosphate pH 6.0. Flow rate was at 0.5 ml/min and the column was kept at ambient temperature. Absorbance at 280 nm was monitored where PEG contributed little signal. Biorad MW standards containing cyanocobalamin, myoglobin, ovalbumin, IgG, Thyroglobulin monomer and dimer were used to generate a standard curve from which the effective size of the pegylated species was estimated.

20 **SEC-HPLC-Light Scattering:** For determination of the exact molecular weight, this column was connected to an on-line light scattering detector (Wyatt Minidawn) equipped with three detection angles of 50°, 90°, and 135° C. A refractive index detector (Wyatt) was also placed on-line to determine concentration. All buffers were filtered with Millipore 0.1 µ filters; in addition a 0.02 µ Whatman Anodisc 25 47 was placed on-line prior to the column.

The intensity of scattered light is directly proportional to the molecular weight (M) of the scattering species, independent of shape, according to:

$$M = R_0 / K \cdot c$$

where R_0 is the Rayleigh ratio, K is an optical constant relating to the refractive index of the solvent, the wavelength of the incident light, and dn/dc , the differential refractive index between the solvent and the solute with respect to the change in solute concentration, c . The system was calibrated with toluene (R_0 of 1.406×10^{-5} at 632.8 nm); a dn/dc of 0.18, and an extinction coefficient of 1.2 was used. The system had a mass accuracy of ~5%.

35 **SDS-PAGE:** 4-12% Tris-Glycine Novex minigels were used along with the Novex supplied Tris-Glycine running buffers. 10-20 µg of protein was applied in each well and the gels were run in a cold box at 150 mV/gel for 45 minutes. Gels were then stained with colloidal Coomassie Blue (Novex) and then washed with water for a few hours and then preserved and dried in drying buffer (Novex)

Preparation of a linear(1)20KDa-(N)-(Fab')₂: A 4 mg/ml solution of anti-IL8 formulated initially in a pH 5.5 buffer was dialyzed overnight against a pH 8.0 sodium phosphate buffer. 5 mL protein

was mixed at a molar ratio of 3:1. The reaction was carried out in a 15mL polypropylene Falcon tube and the PEG was added while vortexing the sample at low speed for 5 seconds. It was then placed on a nutator for 30 minutes. The extent of modification was evaluated by SDS-PAGE. The whole 5 ml reaction mixture was injected on the IEX for removal of any unreacted PEG and purification of singly or doubly pegylated species. The above reaction generated a mixture of 50% singly-labeled anti-IL8. The other 50% unreacted anti-IL8 was recycled through the pegylation/purification steps. The pooled pegylated product was dialyzed against a pH 5.5 buffer for in vitro assays and animal PK studies. Endotoxin levels were measured before administration to animals or for the cell based assays. Levels were below 0.5 eu/ml. The fractions were also run on SDS-PAGE to confirm homogeneity. Concentration of the final product was assessed by absorbance at 280 nm using an extinction coefficient of 1.34, as well as by amino acid analysis.

Preparation of a branched(1)40KDa-(N)-(Fab')₂: A 4 mg/mL solution of anti-IL8 (Fab')₂ formulated in a pH 5.5 buffer was dialyzed overnight against a pH 8.0 phosphate buffer. Solid PEG powder was added to 5 mL protein in two aliquots to give a final PEG:protein molar ratio of 6:1. Each solid PEG aliquot was added to the protein in a 15 mL polypropylene Falcon tube while vortexing at low speed for 5 sec, and then placing the sample on a nutator for 15 minutes. The extent of modification was evaluated by SDS-PAGE using a 4-12% Tris-Glycine (Novex) gel and stained with colloidal Coomassie blue (Novex). The 5 mL PEG-protein mixture was injected on the ion exchange column for removal of any unreacted PEG. The above reaction generated a mixture of unreacted (37%), singly-labelled (45%), doubly and triply-labeled (18%) species. These were the optimal conditions for obtaining the greatest recovery of the protein with only 1 PEG per antibody rather than the higher molecular weight adducts. The unmodified anti-IL8 was recycled. The pegylated products were separated and fractionated in falcon tubes and then dialyzed against a pH 5.5 buffer for assays and animal PK studies. Endotoxin levels were below 0.5 eu/ml. The fractions were also run on SDS-PAGE to confirm homogeneity. The concentration of the final product was assessed by absorbance at 280 nm using an extinction coefficient of 1.34, as well as by amino acid analysis.

Preparation of branched(2)-40KDa-(N)-(Fab')₂: This molecule was most efficiently made by adding three times in 15 minute intervals a 3:1 molar ratio of PEG to the already modified branched(1)-40KDa-(N)-(Fab')₂. The molecule was purified on IEX as 50% branched(2)-40KDa-(N)-(Fab')₂. The unmodified molecule was recycled until ~20 mg protein was isolated for animal PK studies. The product was characterized by SEC-light scattering and SDS-PAGE.

c. RESULTS

PEGs increased the hydrodynamic or effective size of the product significantly as determined by gel filtration (SEC-HPLC). Figure 62 shows the SEC profile of the pegylated F(ab')₂ species with UV detection at 280 nm. The hydrodynamic size of each molecule was estimated by reference to the standard MW calibrators. As summarized in Figure 62, the increase in the effective size of (Fab')₂ was about 7-fold

by adding one linear 20 kDa PEG molecule and about 11-fold by adding one branched ("Br(1)") 40 kDa PEG molecule, and somewhat more with addition of two branched ("Br(2)") PEG molecules.

Light scattering detection gave the exact molecular weight of the products and confirmed the extent of modification (Figure 63). The homogeneity of the purified material was shown by SDS-PAGE (Figure 64). Underivatized F(ab')₂ migrated as a 120 kDa species, the linear(1)20KD-(N)-F(ab')₂ migrated as a band at 220kDa, the Br(1)-40KD(N)-F(ab')₂ migrated as one major band at 400 kDa, and the Br(2)-40KD-(N)-F(ab')₂ migrated as a major band at around 500 kDa. The proteins appeared somewhat larger than their absolute MW due to the steric effect of PEG.

V. IN VITRO ACTIVITY CHARACTERIZATION OF PEG MODIFIED Fab' FRAGMENTS OF 6G4V11N35A (MALEIMIDE CHEMICAL COUPLING METHOD)

Anti-IL-8 6G4V11N35A Fab' variants modified with 5-40kD linear PEG molecules and a 40kD branched PEG molecule were tested for their ability to inhibit both IL-8 binding and activation of human neutrophils; the procedures were described in Sections (B)(1), (B)(2) and (B)(3) above. The binding curves and IC₅₀'s for PEG-maleimide modified 6G4V11N35A Fab' molecules are presented in Figures 54A-54C.

The IC₅₀ of the 5kD pegylated Fab' (350pM) and the average IC₅₀ of the Fab control (366pM) were not significantly different, suggesting that the addition of a 5kD MW PEG did not affect the binding of IL-8 to the modified Fab' (Figure 54A). However, a decrease in the binding of IL-8 to the 10kD- and 20kD pegylated Fab' molecules was observed as depicted by the progressively higher IC₅₀'s (537pM and 732pM, respectively) compared to the average IC₅₀ of the native Fab. These values represent only a minimal loss of binding activity (between 1.5- and 2.0-fold). A less pronounced difference in IL-8 binding was observed for the 30kD and 40kD linear PEG antibodies (Figure 54B). The IC₅₀'s were 624pM and 1.1nM, respectively, compared to the 802pM value of the Fab control. The 40kD branched PEG Fab' showed the largest decrease in IL-8 binding (2.5 fold) relative to the native Fab (Figure 54C). Nevertheless, the reduction in binding of IL-8 by these pegylated Fab's is minimal.

The ability of the pegylated antibodies to block IL-8 mediated activation of human neutrophils was demonstrated using the PMN chemotaxis (according to the method described in Section B(2) above) and β -glucuronidase release (according to the method described in Lowman et al., *J. Biol. Chem.*, 271: 14344 (1996)) assays. The IC₅₀'s for blocking IL-8 mediated chemotaxis are shown in Figures 55A-55C. The 5-20kD linear pegylated Fab' antibodies were able to block IL-8 mediated chemotaxis within 2-3 fold of the unpegylated Fab control (Figure 55A). This difference is not significant because the inherent variation can be up to 2 fold for this type of assay. However, a significant difference was detected for the 30kD and 40kD linear pegylated Fab' antibodies as illustrated by the higher IC₅₀'s of the 30kD linear PEG-Fab' (2.5nM) and 40kD linear-PEG-Fab' (3.7nM) compared to the Fab control (0.8nM) (Figure 55B).

The ability of the 40kD branch d PEG Fab' molecule to block IL-8 mediated chemotaxis was similar to that of the 40kD linear PEG Fab' (Figure 55C). At most, the ability of the pegylated Fab' antibodies to block IL-8 mediated chemotaxis was only reduced 2-3 fold. Furthermore, release of β -glucuronidase from the granules of neutrophils was used as another criteria for assessing IL-8 mediated activation of human PMNs. Figure 56A (depicting results obtained with 5 kD, 10 kD and 20 kD linear PEGs), Figure 56B (depicting results obtained with 30 kD and 40 kD linear PEGs), and Figure 56C (depicting results obtained with 40 kD branched PEG) show that all the pegylated Fab' antibodies were able to inhibit IL-8 mediated release of β -glucuronidase as well as or better than the unpegylated Fab control. The data collectively shows that the pegylated Fab' variants are biological active and are capable of inhibiting high amounts of exogenous IL-8 in in-vitro assays using human neutrophils.

W. IN VITRO ACTIVITY CHARACTERIZATION OF PEG MODIFIED F(ab')₂ FRAGMENTS OF 6G4V11N35A (SUCCINIMIDYL CHEMICAL COUPLING METHOD)

The anti-IL-8 variant 6G4V11N35A F(ab')₂ modified with (a) a single 20kD linear PEG molecule per F(ab')₂, (b) a single 40kD branched PEG molecule per F(ab')₂, (c) with three, four, or five 20 kD linear PEG molecules per F(ab')₂ (a mixture of: (1) species having three 20 kD linear PEG molecules per F(ab')₂; (2) species having four 20 kD linear PEG molecules per F(ab')₂; and (3) species having five 20 kD linear PEG molecules per F(ab')₂; denoted as "20 kD linear PEG (3,4,5) F(ab')₂"), or (d) with two 40kD branched PEG molecules per F(ab')₂ (denoted as "40 kD branch PEG (2) F(ab')₂"), were tested for their ability to inhibit ¹²⁵I-IL-8 binding and to neutralize activation of human neutrophils. The procedures used are described in Sections (B)(1), (B)(2) and (B)(3) above. The binding curves for pegylated F(ab')₂ variants are shown in Figures 57A-57B. No significant differences were observed amongst the F(ab')₂ control, the single 20kD linear PEG-modified F(ab')₂, and the single 40kD branched PEG-modified F(ab')₂ (Figure 57A). However, the F(ab')₂ variants containing multiple PEG molecules showed a slight reduction (less than 2-fold) in their ability to bind IL-8. The IC₅₀'s of the 20kD linear PEG (3,4,5) F(ab')₂ and 40kD branch PEG (2) F(ab')₂ variants were 437pM and 510pM, respectively, compared to 349pM of the F(ab')₂ control (Figure 57B).

The ability of these pegylated F(ab')₂ variants to block IL-8 mediated neutrophil chemotaxis is presented in Figures 58A-58B. Consistent with the PMN binding data, the single linear and branched PEG F(ab')₂ variants were able to block IL-8 mediated chemotaxis similar to the unpegylated F(ab')₂ control (Figure 58A). The ability of the 40kD branch PEG (2) F(ab')₂ variant to inhibit PMN chem taxis was

identical to the control $F(ab')_2$ while the 20kD linear PEG (3,4,5) $F(ab')_2$ mixture was able to inhibit within 3-fold of the control antibody (Figure 58B).

Shown in Figures 59A and 59B are the results of the β -glucuronidase release assay which is a measure of degranulation by IL-8 stimulated human neutrophils. The single 20kD linear PEG-modified $F(ab')_2$ and the single 40kD branched PEG-modified $F(ab')_2$ variants were able to inhibit release of β -glucuronidase as well as the $F(ab')_2$ control (Figure 59A). The 40kD branch PEG (2) $F(ab')_2$ inhibited this response within 2-fold of the $F(ab')_2$ control (Figure 59B). The 20kD linear PEG (3,4,5) molecule was not tested. Overall, the $F(ab')_2$ pegylated anti-IL-8 antibodies were biologically active and effectively prevented IL-8 binding to human neutrophils and the signaling events leading to cellular activation.

10 X. PHARMACOKINETIC AND SAFETY STUDY OF EIGHT CONSTRUCTS OF PEGYLATED ANTI-IL-8 (HUMANIZED) $F(ab')_2$ AND Fab' FRAGMENTS IN NORMAL RABBITS FOLLOWING INTRAVENOUS ADMINISTRATION

The objective of this study was to evaluate the effect of pegylation on the pharmacokinetics and safety of six pegylated humanized anti-IL-8 constructs (pegylated 6G4V11N35A. Fab' and pegylated 6G4V11N35A. $F(ab')_2$ obtained as described in Sections (T) and (U) above) relative to the non-pegylated fragments in normal rabbits. Eight groups of two/three male rabbits received equivalent protein amounts of pegylated 6G4V11N35A. Fab' or pegylated 6G4V11N35A. $F(ab')_2$ constructs (2 mg/kg) via a single intravenous (IV) bolus dose of one anti-IL8 construct. Serum samples were collected according to the schedule shown in Table 8 below and analyzed for anti-IL8 protein concentrations and antibody formation against anti-IL8 constructs by ELISA.

Table 8

Group No.	Dose level/ Route	Material	Blood Collection
1	2 mg/kg (protein conc.) IV bolus	Fab' control	0,5,30 min; 1,2,3,4,6,8,10, 14,20,24,360 hr
2		linear(1)20K(s) Fab'	0,5,30 min; 1,2,4,6,8,10,12, 24,28,32,48,72,96,168,216, 264,336,360 hr
3		linear(1)40K(s) Fab'	
4		branched(1)40K(N) $F(ab')_2$	
5		$F(ab')_2$ control	0,5,30 min; 1,2,4,6,8,10,12, 24,28,32,48,52,56,336 hr

Group No.	Dose level/ Route	Material	Blood Collection
6		branched(2)40K(s)Fab'	0,5,30 min; 1,2,4,6,8,10,12, 24,28,32,48,72,96,168,216,264,3 36 hr; Day 17,21, 25
7		branched(2)40K(N)F(ab') ₂	0,5,30 min; 1,2,4,6,8,10,12, 24,28,32,48,72,144,192, 240 hr; Day 13, 16, 20, 23
8		linear(1)30K(s)Fab'	0,5,30 min; 1,2,4,6,8,10,12, 24,28,32,48,72,96,168,216,264,3 36 hr; Day 17,21, 25

a. METHODS

Three male New Zealand White (NZW) rabbits per group (with exception to Group 7, n=2) received an equivalent amount of 6G4V11N35A protein (Fab' or F(ab')₂) construct at 2 mg/kg via an IV bolus dose in a marginal ear vein. Amino acid composition analysis and absorbance at 280 nm using extinction coefficients of 1.26 for 6G4V11N35A Fab' constructs and 1.34 for 6G4V11N35A F(ab')₂ constructs were performed to determine the protein concentration. Whole blood samples were collected via an ear artery cannulation (ear opposing dosing ear) at the above time points. Samples were harvested for serum and assayed for free 6G4V11N35A Fab' or F(ab')₂ constructs using an IL-8 Binding ELISA. Assays were conducted throughout the study as samples became available. All animals were sacrificed following the last blood draw, and necropsies were performed on all animals in Groups 1, 4–8. Due to the development of antibodies against the 6G4V11N35A constructs, non-compartmental pharmacokinetic analysis was conducted on concentration versus time data only up to 168 hours.

b. RESULTS

In four animals (Animals B, P, Q, V), interference to rabbit serum in the ELISA assay was detected (i.e. measurable concentrations of anti-IL8 antibodies at pre-dose). However, because these values were at insignificant levels and did not effect the pharmacokinetic analysis, the data were not corrected for this interference.

One animal (Animal G; Group 3) was exsanguinated before the termination of the study and was excluded from the pharmacokinetic analysis. At 4 hours, the animal showed signs of a stroke that was not believed to be drug related, as this can occur in rabbits following blood draws via ear artery cannulation.

The mean concentration–time profiles of the eight anti-IL8 constructs in normal rabbits are depicted in Fig. 65, and the pharmacokinetic parameters for the eight constructs are summarized in Table 9 below. Significant antibodies to the anti-IL-8 constructs were present at Day 13/14 in all dose groups except Group 1 (Fab' control).

Table 9. Pharmacokinetic parameters.

Molecule	Fab'					F(ab') ₂		
Group No.	1	2	8	3	6	5	4	7
PEG structure	—	linear	linear	linear	branched	—	branched	branched
Number of PEGs	—	1	1	1	1	—	1	2
PEG MW	—	20K	30K	40K	40K	—	40K	40K
Dose (mg/kg)	2	2	2	2	2	2	2	2
V _c (mL/kg) ^a	58±3	36±3	35±1	34	44±1	45±5	36±1	32
V _{ss} (mL/kg) ^b	68±8	80±8	110±15	79	88±21	59±4	50±3	52
C _{max} (µg/mL) ^c	35±1	58±3	57±1	60	45±1	45±6	56±2	62
T _{max} (min) ^d	5	5	5	5	5	5	5	5
t _{1/2} term (hr) ^e	3.0±0.9	44±2	43±7	50	105±11	8.5±2.1	45±3	48
AUC _{0-∞} (hr·µg/mL) ^f	18±3	80±74	910±140	1600	3400±1300	140±3	2200±77	2500
CL (mL/hr/kg) ^g	110±17	2.5±0.2	2.2±0.4	1.3	0.63±0.20	14±0	0.92±0.03	0.83
MRT (hr) ^h	0.61±0.15	32±2	45±9	63	140±18	4.2±0.3	55±3	64
No. of Animals	3	3	3	2	3	3	3	2

^a Initial volume of distribution.

^b Volume of distribution at steady state.

^c Observed maximum concentration.

^d Observed time to C_{max}.

^e t_{1/2} term = half-life associated with the terminal phase of the concentration vs. time profile.

^f Area under the concentration versus time curve (extrapolated to infinity).

^g CL = serum clearance.

^h MRT = Mean residence time.

10 The initial volume of distribution approximated the plasma volume for both the Fab' and F(ab')₂.
 Pegylation decreased serum CL of anti-IL8 fragments and extended both the terminal half-life and MRT as shown in Table 10 below.

Table 10. Fold decrease/increase in clearance, terminal half-life & MRT of pegylated anti-IL8 fragments.

anti-IL8 fragment		Fab'					F(ab') ₂		
Group No.		1	2	8	3	6	5	4	7
PEG structure		—	linear	linear	linear	bran.	—	bran.	bran.
No. of PEGs		—	1	1	1	1	—	1	2
PEG MW		—	20K	30K	40K	40K	—	40K	40K
CL :	mean (mL/hr/kg)	110	2.5	2.2	1.3	0.63	14	0.92	0.83
	fold decrease	1	46	51	90	180	1	15	17
t _{1/2} term :	mean (hr)	3.0	44	43	50	110	8.5	45	48
	fold increase	1	14	14	17	35	1	5.3	5.7
MRT :	mean (hr)	0.61	32	45	63	140	4.2	55	64
	fold increase	1	53	73	100	240	1	13	15

For the pegylated anti-IL8 Fab' fragments, CL decreased by 46 to 180-fold. Terminal half-life and MRT increased 14 to 35-fold and 53 to 240-fold, respectively. For pegylated anti-IL8 F(ab')₂ molecules, CL decreased 15 to 17-fold with pegylation, and terminal half-life and MRT increased by greater than 5-fold and 13-fold, respectively. The changes in these parameters increased for both pegylated Fab' and F(ab')₂ molecules with increasing PEG molecular weight and approached the values of the full-length anti-IL8 (terminal half-life of 74 hours, MRT of 99 hours and CL of 0.47 mL/hr/kg). In comparing the branched(1)40K Fab' (Group 6) and branched(1)40K F(ab')₂ (Group 4), unexpected pharmacokinetics were observed. The pegylated Fab' molecule appeared to remain in the serum longer than the pegylated F(ab')₂ (see Figure 66). The mean CL of branched(1)40K Fab' was 0.63 mL/hr/kg, but a higher CL was observed for branched(1)40kD F(ab')₂ (CL 0.92 mL/hr/kg). The terminal half-life, likewise, was longer for the Fab' than the F(ab')₂ pegylated molecule (110 vs 45 hours).

The pharmacokinetic data demonstrated that pegylation decreased CL and increased terminal t_{1/2} and MRT of anti-IL8 fragments (Fab' and F(ab')₂) to approach that of the full-length anti-IL8. Clearance was decreased with pegylation 46 to 180-fold for the Fab' and approximately 16-fold for the F(ab')₂. The terminal half-life of the Fab' anti-IL8 fragment was increased by 14 to 35-fold and approximately 5-fold for the F(ab')₂ anti-IL8. MRT, likewise, were extended by 53 to 240-fold for the Fab' and approximately 14-fold for the F(ab')₂. The branched(1) 40kD Fab' had a longer terminal half-life and lower clearance compared to the branched(1) 40kD F(ab')₂.

Y. IN VIVO EFFICACY TESTING OF ANTI-IL-8 ANTIBODY REAGENTS IN RABBIT MODEL OF ISCHEMIA/REPERFUSION AND ACID ASPIRATION-INDUCED ACUTE RESPIRATORY DISTRESS SYNDROME (ARDS)

Full length murine anti-rabbit IL-8 monoclonal antibody 6G4.2.5, 40 kD branched PEG-6G4V11N35A Fab', and control antibody (anti-HIV gp120 monoclonal antibody 9E3.1F10) were tested in a rabbit ARDS model. The animals were weighed and anaesthetized by intramuscular injection of ketamine (50 mg/kg body weight), xylazine (5 mg/kg body weight), and acepromazine (0.75 mg/kg body weight). A second dose (20% of the first dosage) was given IM 15 minutes before removal of vascular clip, and third dose (60% of the first dosage) was given at tracheotomy. Intra-arterial catheter (22G, 1 in. Angiocath) and intra-venous catheter (24G, 1 in. angiocath) were placed in the ear central artery and posterior marginal ear vein for blood samplings (arterial blood gases and CBC) and anti-IL-8 and fluid administration, respectively. The anaesthetized animals were transferred in a supine position to an operating tray; the abdominal area was shaved and prepared for surgery. Via a midline laparotomy, the superior mesenteric artery (SMA) was isolated and a microvascular arterial clip applied at the aortic origin. Before the temporary closure of the abdomen using 9 mm wound clip (Autoclip, Baxter), 15 ml of normal saline was

given intraperitoneally as fluid supplement. After 110 minutes of intestinal ischemia, the abdominal incision was reopened and the arterial clip was released to allow reperfusion. Before closure, 5 ml of normal saline was given intraperitoneally for fluid replacement. The laparotomy incision was closed in two layers and the animals allowed to awaken.

5 After surgery, the animals were placed on a heating pad (38°C) and continuously monitored for up to 6 hours post reperfusion and lactated Ringer's 8-12 ml/kg/hr IV was given as fluid supplement.

At 22-24 hr post-reperfusion, a tracheotomy was performed under anesthesia. Normal physiologic saline was diluted 1:3 with water and adjusted to pH 7.5 (adjusted by using 1N HCL); 3 ml/kg body weight was then instilled intra-tracheally. Rectal temperature was maintained at 37 +/- 1 degree C using a homeothermic heat therapy pad (K-Mod II, Baxter). Fluid supplements (LRS) at a rate of 5 ml/kg/hour IV were given. Blood gases were monitored every hour. The rabbits were returned to the cage after 6 hr of continuous monitoring.

Just prior to aspiration, animals were treated with saline, the control monoclonal antibody (anti-HIV gp-120 IgG 9E3.1F10), the full length murine anti-rabbit IL8 (6g4.2.5 murine IgG2a anti-rabbit IL8) or the pegylated 6G4V11N35A Fab' (6G4V11N35A Fab' modified with 40kD branched PEG-maleimide as described in Section T above, denoted as "40 kD branched PEG-6G4V11N35A Fab' "). Data from saline or control antibody treated animals was combined and presented as "Control". Arterial blood gases and A-a PO2 gradient measurements were taken daily, and IV fluid supplementation was performed daily. A-a PO2 gradient was measured at 96 hr of reperfusion. The A-a PO2 gradient was calculated as:

20
$$A-a\ PO_2 = [FIO_2(PB - PH_2O) - (PaCO_2/RQ)] - PaO_2.$$

PaO2/FiO2 ratios were measured at 24hr and 48hr in room air and 100% oxygen.

After the final A-a PO2 gradient measurement, the animals were anesthetized with Nembutal 100mg/kg i.v. and the animals were euthanized by transecting the abdominal aorta in order to reduce red blood cell contamination of bronchoalveolar lavage fluid (BAL). The lungs were removed en bloc. The entire lung was weighed and then lavaged with an intratracheal tube (Hi-Lo tracheal tube, 3mm) using 30 ml of HBSS and lidocain. Total and differential leukocyte counts in the BAL were determined. Lesions/changes were verified by histological examination of each lobe of the right lung of each animal.

The gross lung weight, total leukocyte and polymorphonuclear cell counts in BAL, and PaO2/FiO2 data obtained are depicted in Figs. 67, 68 and 69, respectively. Treatment with 40 kD branched PEG-6G4V11N35A Fab' exhibited no effect on the biological parameters measured in the model as compared to the "Control" group. However, the data do not contradict the pharmacokinetic analysis or the in vitro activity analysis for the 40 kD branched PEG-6G4V11N35A Fab' presented in Sections (V) and (X) above. In addition, these data do not contradict the ability of the 40 kD branched PEG-6G4V11N35A Fab' to reach and act on disease effector targets in circulation or other tissues.

35 The following biological materials have been deposited with the American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD, USA (ATCC):

	<u>Material</u>	<u>ATCC Accession No.</u>	<u>Deposit Date</u>
	hybridoma cell line 5.12.14	HB 11553	February 15, 1993
	hybridoma cell line 6G4.2.5	HB 11722	September 28, 1994
5	pantIL-8.2, E. coli strain 294 mm	97056	February 10, 1995
	p6G425chim2, E. coli strain 294 mm	97055	February 10, 1995
	p6G4V11N35A.F(ab') ₂	97890	February 20, 1997
	E. coli strain 49D6(p6G4V11N35A.F(ab') ₂)	98332	February 20, 1997
	p6G425V11N35A.choSD	209552	December 16, 1997
10	clone#1933 aIL8.92 NB 28605/12	CRL-12444	December 11, 1997
	clone#1934 aIL8.42 NB 28605/14	CRL-12445	December 11, 1997

These deposits were made under the provisions of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purpose of Patent Procedure and the Regulations thereunder (Budapest Treaty). This assures maintenance of a viable deposit for 30 years from the date of deposit. These cell lines will be made available by ATCC under the terms of the Budapest Treaty, and subject to an agreement between Genentech, Inc. and ATCC, which assures permanent and unrestricted availability of the cell lines to the public upon issuance of the pertinent U.S. patent or upon laying open to the public of any U.S. or foreign patent application, whichever comes first, and assures availability of the cell lines to one determined by the U.S. Commissioner of Patents and Trademarks to be entitled thereto according to 35 USC §122 and the Commissioner's rules pursuant thereto (including 37 CFR §1.14 with particular reference to 886 OG 638).

The assignee of the present application has agreed that if the deposited cell lines should be lost or destroyed when cultivated under suitable conditions, they will be promptly replaced on notification with a specimen of the same cell line. Availability of the deposited cell lines is not to be construed as a license to practice the invention in contravention of the rights granted under the authority of any government in accordance with its patent laws

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- (ii) TITLE OF INVENTION: Antibody Fragment-Polymer Conjugates and
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- 15 (iii) NUMBER OF SEQUENCES: 76
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- 25 (v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: 3.5 inch, 1.44 Mb floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
30 (D) SOFTWARE: WinPatin (Genentech)
- (vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
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35 (C) CLASSIFICATION:
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- 45 (2) INFORMATION FOR SEQ ID NO:1:
- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: Nucleic Acid
50 (C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:
- 55

CAGTCCAAC T GTTCAGGACG CC 22

(2) INFORMATION FOR SEQ ID NO:2:

- 5 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 22 base pairs
 (B) TYPE: Nucleic Acid
 (C) STRANDEDNESS: Single
10 (D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

GTGCTGCTCA TGCTGTAGGT GC 22

15

(2) INFORMATION FOR SEQ ID NO:3:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 23 base pairs
20 (B) TYPE: Nucleic Acid
 (C) STRANDEDNESS: Single
 (D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

25

GAAGTTGATG TCTTGTGAGT GGC 23

(2) INFORMATION FOR SEQ ID NO:4:

30

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 24 base pairs
 (B) TYPE: Nucleic Acid
 (C) STRANDEDNESS: Single
35 (D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

40 GCATCCTAGA GTCACCGAGG AGCC 24

(2) INFORMATION FOR SEQ ID NO:5:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 22 base pairs
45 (B) TYPE: Nucleic Acid
 (C) STRANDEDNESS: Single
 (D) TOPOLOGY: Linear

50 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

CACTGGCTCA GGGAAATAAC CC 22

55 (2) INFORMATION FOR SEQ ID NO:6:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 bas pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:
- GGAGAGCTGG GAAGGTGTGC AC 22
- (2) INFORMATION FOR SEQ ID NO:7:
- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 35 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:
- ACAAACGCGT ACGCTGACAT CGTCATGACC CAGTC 35
- (2) INFORMATION FOR SEQ ID NO:8:
- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 35 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:
- ACAAACGCGT ACGCTGATAT TGTCATGACT CAGTC 35
- (2) INFORMATION FOR SEQ ID NO:9:
- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 35 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:
- ACAAACGCGT ACGCTGACAT CGTCATGACA CAGTC 35
- (2) INFORMATION FOR SEQ ID NO:10:
- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 37 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

5

GCTCTTCGAA TGGTGGGAAG ATGGATACAG TTGGTGC 37

(2) INFORMATION FOR SEQ ID NO:11:

10

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 39 base pairs

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

15

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

CGATGGGCCC GGATAGACCG ATGGGGCTGT TGTTTTGGC 39

20

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 39 base pairs

25

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

30

CGATGGGCCC GGATAGACTG ATGGGGCTGT CGTTTTGGC 39

(2) INFORMATION FOR SEQ ID NO:13:

35

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 39 base pairs

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

40

(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

45

CGATGGGCCC GGATAGACGG ATGGGGCTGT TGTTTTGGC 39

(2) INFORMATION FOR SEQ ID NO:14:

50

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 39 base pairs

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

55

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

CGATGGGCCC GGATAGACAG ATGGGGCTGT TGTTTTGGC 39

(2) INFORMATION FOR SEQ ID NO:15:

5

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 39 base pairs

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

10

(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

15 CGATGGGCCC GGATAGACCG ATGGGGCTGT TGTTTTGGC 39

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 39 base pairs

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

20

(D) TOPOLOGY: Linear

25 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

CGATGGGCCC GGATAGACTG ATGGGGCTGT TGTTTTGGC 39

30 (2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 39 base pairs

(B) TYPE: Nucleic Acid

35

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

40

CGATGGGCCC GGATAGACAG ATGGGGCTGT TGTTTTGGC 39

(2) INFORMATION FOR SEQ ID NO:18:

45 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 39 base pairs

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

50

(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

CGATGGGCCC GGATAGACGG ATGGGGCTGT TGTTTTGGC 39

55

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH: 369 base pairs
 (B) TYPE: Nucleic Acid
 (C) STRANDEDNESS: Double
 (D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

10 GACATTGTCA TGACACAGTC TCAAAAATTC ATGTCCACAT CAGTAGGAGA 50
 CAGGGTCAGC GTCACCTGCA AGGCCAGTCA GAATGTGGGT ACTAATGTAG 100
 15 CCTGGTATCA ACAGAAACCA GGGCAATCTC CTAAAGCACT GATTTACTCG 150
 TCATCCTACC GGTACAGTGG AGTCCCTGAT CGCTTCACAG GCAGTGGATC 200
 TGGGACAGAT TTCACTCTCA CCATCAGCCA TGTGCAGTCT GAAGACTTGG 250
 20 CAGACTATTT CTGTCAGCAA TATAACATCT ATCCTCTCAC GTTCGGTCCT 300
 GGGACCAAGC TGGAGTTGAA ACGGGCTGAT GCTGCACCAC CAACTGTATC 350
 25 CATCTTCCCA CCATTCGAA 369

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:

- 30 (A) LENGTH: 123 amino acids
 (B) TYPE: Amino Acid
 (D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

35 Asp Ile Val Met Thr Gln Ser Gln Lys Phe Met Ser Thr Ser Val
 1 5 10 15
 40 Gly Asp Arg Val Ser Val Thr Cys Lys Ala Ser Gln Asn Val Gly
 20 25 30
 Thr Asn Val Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ser Pro Lys
 35 40 45
 45 Ala Leu Ile Tyr Ser Ser Ser Tyr Arg Tyr Ser Gly Val Pro Asp
 50 55 60
 Arg Phe Thr Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile
 65 70 75
 50 Ser His Val Gln Ser Glu Asp Leu Ala Asp Tyr Phe Cys Gln Gln
 80 85 90
 55 Tyr Asn Ile Tyr Pro Leu Thr Phe Gly Pro Gly Thr Lys Leu Glu
 95 100 105

Leu Lys Arg Ala Asp Ala Ala Pro Pro Thr Val Ser Ile Phe Pro
 110 115 120

Pro Phe Glu
 123

5

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:

- 10 (A) LENGTH: 417 base pairs
 (B) TYPE: Nucleic Acid
 (C) STRANDEDNESS: Double
 (D) TOPOLOGY: Linear

15 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

TTCTATTGCT ACAAACGCGT ACGCTGAGGT GCAGCTGGTG GAGTCTGGGG 50
 20 GAGGCTTAGT GCCGCCTGGA GGGTCCCTGA AACTCTCCTG TGCAGCCTCT 100
 GGATTCATAT TCAGTAGTTA TGGCATGTCT TGGGTTTCGCC AGACTCCAGG 150
 CAAGAGCCTG GAGTTGGTCG CAACCATTAA TAATAATGGT GATAGCACCT 200
 25 ATTATCCAGA CAGTGTGAAG GGCCGATTCA CCATCTCCCG AGACAATGCC 250
 AAGAACACCC TGTACCTGCA AATGAGCAGT CTGAAGTCTG AGGACACAGC 300
 30 CATGTTTTAC TGTGCAAGAG CCCTCATTAG TTCGGCTACT TGGTTTGGTT 350
 ACTGGGGCCA AGGGACTCTG GTCAGTGTCT CTGCAGCCAA AACAACAGCC 400
 CCATCTGTCT ATCCGGG 417

35

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:

- 40 (A) LENGTH: 130 amino acids
 (B) TYPE: Amino Acid
 (D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

45 Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Pro Pro Gly
 1 5 10 15
 Gly Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Ile Phe Ser
 20 25 30
 50 Ser Tyr Gly Met Ser Trp Val Arg Gln Thr Pro Gly Lys Ser Leu
 35 40 45
 Glu Leu Val Ala Thr Ile Asn Asn Asn Gly Asp Ser Thr Tyr Tyr
 55 50 55 60

Pro Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala
65 70 75
5 Lys Asn Thr Leu Tyr Leu Gln Met Ser Ser Leu Lys Ser Glu Asp
80 85 90
Thr Ala Met Phe Tyr Cys Ala Arg Ala Leu Ile Ser Ser Ala Thr
95 100 105
10 Trp Phe Gly Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ala
110 115 120
Ala Lys Thr Thr Ala Pro Ser Val Tyr Pro
125 130
15

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
20 (A) LENGTH: 31 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:
25

ACAAACGCGT ACGCTGATAT CGTCATGACA G 31

(2) INFORMATION FOR SEQ ID NO:24:
30

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
35 (D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

GCAGCATCAG CTCTTCGAAG CTCCAGCTTG G 31

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
45 (A) LENGTH: 21 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

CCACTAGTAC GCAAGTTCAC G 21

(2) INFORMATION FOR SEQ ID NO:26:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

5

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

10 GATGGGCCCT TGGTGGAGGC TGCAGAGACA GTG 33

(2) INFORMATION FOR SEQ ID NO:27:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 714 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Double
(D) TOPOLOGY: Linear

15

20 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

ATGAAGAAGA ATATCGCATT TCTTCTTGCA TCTATGTTTCG TTTTCTCTAT 50
25 TGCTACAAAC GCGTACGCTG ATATCGTCAT GACACAGTCT CAAAATTCA 100
TGTCCACATC AGTAGGAGAC AGGGTCAGCG TCACCTGCAA GGCCAGTCAG 150
AATGTGGGTA CTAATGTAGC CTGGTATCAA CAGAAACCAG GGCAATCTCC 200
30 TAAAGCACTG ATTTACTCGT CATCCTACCG GTACAGTGGA GTCCCTGATC 250
GCTTCACAGG CAGTGGATCT GGGACAGATT TCACTCTCAC CATCAGCCAT 300
35 GTGCAGTCTG AAGACTTGGC AGACTATTTT TGTCAGCAAT ATAACATCTA 350
TCCTCTCACG TTCGGTCCTG GGACCAAGCT GGAGCTTCGA AGAGCTGTGG 400
CTGCACCATC TGTCTTCATC TTCCCGCCAT CTGATGAGCA GTTGAAATCT 450
40 GGAAGTCTT CTGTTGTGTG CCTGCTGAAT AACTTCTATC CCAGAGAGGC 500
CAAAGTACAG TGAAGGTGG ATAACGCCCT CCAATCGGGT AACTCCCAGG 550
45 AGAGTGTAC AGAGCAGGAC AGCAAGGACA GCACCTACAG CCTCAGCAGC 600
ACCCTGACGC TGAGCAAAGC AGACTACGAG AAACACAAAG TCTACGCCTG 650
CGAAGTCACC CATCAGGGCC TGAGCTCGCC CGTCACAAAG AGCTTCAACA 700
50 GGGGAGAGTG TTAA 714

(2) INFORMATION FOR SEQ ID NO:28:

- 55 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 237 amino acids

(B) TYPE: Amino Acid

(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

5	Met	Lys	Lys	Asn	Ile	Ala	Phe	Leu	Leu	Ala	Ser	Met	Phe	Val	Phe
	1				5					10					15
10	Ser	Ile	Ala	Thr	Asn	Ala	Tyr	Ala	Asp	Ile	Val	Met	Thr	Gln	Ser
					20					25					30
	Gln	Lys	Phe	Met	Ser	Thr	Ser	Val	Gly	Asp	Arg	Val	Ser	Val	Thr
					35					40					45
15	Cys	Lys	Ala	Ser	Gln	Asn	Val	Gly	Thr	Asn	Val	Ala	Trp	Tyr	Gln
					50					55					60
	Gln	Lys	Pro	Gly	Gln	Ser	Pro	Lys	Ala	Leu	Ile	Tyr	Ser	Ser	Ser
					65					70					75
20	Tyr	Arg	Tyr	Ser	Gly	Val	Pro	Asp	Arg	Phe	Thr	Gly	Ser	Gly	Ser
					80					85					90
	Gly	Thr	Asp	Phe	Thr	Leu	Thr	Ile	Ser	His	Val	Gln	Ser	Glu	Asp
					95					100					105
25	Leu	Ala	Asp	Tyr	Phe	Cys	Gln	Gln	Tyr	Asn	Ile	Tyr	Pro	Leu	Thr
					110					115					120
30	Phe	Gly	Pro	Gly	Thr	Lys	Leu	Glu	Leu	Arg	Arg	Ala	Val	Ala	Ala
					125					130					135
	Pro	Ser	Val	Phe	Ile	Phe	Pro	Pro	Ser	Asp	Glu	Gln	Leu	Lys	Ser
					140					145					150
35	Gly	Thr	Ala	Ser	Val	Val	Cys	Leu	Leu	Asn	Asn	Phe	Tyr	Pro	Arg
					155					160					165
40	Glu	Ala	Lys	Val	Gln	Trp	Lys	Val	Asp	Asn	Ala	Leu	Gln	Ser	Gly
					170					175					180
	Asn	Ser	Gln	Glu	Ser	Val	Thr	Glu	Gln	Asp	Ser	Lys	Asp	Ser	Thr
					185					190					195
45	Tyr	Ser	Leu	Ser	Ser	Thr	Leu	Thr	Leu	Ser	Lys	Ala	Asp	Tyr	Glu
					200					205					210
	Lys	His	Lys	Val	Tyr	Ala	Cys	Glu	Val	Thr	His	Gln	Gly	Leu	Ser
					215					220					225
50	Ser	Pro	Val	Thr	Lys	Ser	Phe	Asn	Arg	Gly	Glu	Cys			
					230					235		237			

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 756 base pairs
 (B) TYPE: Nucleic Acid
 (C) STRANDEDNESS: Double
 (D) TOPOLOGY: Linear

5

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

10 ATGAAAAAGA ATATCGCATT TCTTCTTGCA TCTATGTTCTG TTTTCTCTAT 50
 TGCTACAAAC GCGTACGCTG AGGTGCAGCT GGTGGAGTCT GGGGGAGGCT 100
 TAGTGCCGCC TGGAGGGTCC CTGAAACTCT CCTGTGCAGC CTCTGGATTC 150
 15 ATATTCAGTA GTTATGGCAT GTCTTGGGTT CGCCAGACTC CAGGCAAGAG 200
 CCTGGAGTTG GTCGCAACCA TTAATAATAA TGGTGATAGC ACCTATTATC 250
 CAGACAGTGT GAAGGGCCGA TTCACCATCT CCCGAGACAA TGCCAAGAAC 300
 20 ACCCTGTACC TGCAAATGAG CAGTCTGAAG TCTGAGGACA CAGCCATGTT 350
 TTA CTGTGCA AGAGCCCTCA TTAGTTCGGC TACTTGGTTT GGTTACTGGG 400
 25 GCCAAGGGAC TCTGGTCACT GTCTCTGCAG CCTCCACCAA GGGCCCATCG 450
 GTCTTCCCCC TGGCACCCCTC CTCCAAGAGC ACCTCTGGGG GCACAGCGGC 500
 CCTGGGCTGC CTGGTCAAGG ACTACTTCCC CGAACCGGTG ACGGTGTCGT 550
 30 GGAACTCAGG CGCCCTGACC AGCGGCGTGC ACACCTTCCC GGCTGTCCTA 600
 CAGTCCTCAG GACTCTACTC CCTCAGCAGC GTGGTGACCG TGCCCTCCAG 650
 35 CAGCTTGGGC ACCCAGACCT ACATCTGCAA CGTGAATCAC AAGCCCAGCA 700
 ACACCAAGGT GGACAAGAAA GTTGAGCCCA AATCTTGTGA CAAAACCTCAC 750
 ACATGA 756

40

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:

- 45 (A) LENGTH: 251 amino acids
 (B) TYPE: Amino Acid
 (D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

50 Met Lys Lys Asn Ile Ala Phe Leu Leu Ala Ser Met Phe Val Phe
 1 5 10 15
 Ser Ile Ala Thr Asn Ala Tyr Ala Glu Val Gln Leu Val Glu Ser
 20 25 30
 55 Gly Gly Gly Leu Val Pro Pro Gly Gly Ser Leu Lys Leu Ser Cys

	35	40	45
	Ala Ala Ser Gly Phe Ile Phe Ser Ser Tyr Gly Met Ser Trp Val		
	50	55	60
5	Arg Gln Thr Pro Gly Lys Ser Leu Glu Leu Val Ala Thr Ile Asn		
	65	70	75
	Asn Asn Gly Asp Ser Thr Tyr Tyr Pro Asp Ser Val Lys Gly Arg		
10	80	85	90
	Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr Leu Gln		
	95	100	105
15	Met Ser Ser Leu Lys Ser Glu Asp Thr Ala Met Phe Tyr Cys Ala		
	110	115	120
	Arg Ala Leu Ile Ser Ser Ala Thr Trp Phe Gly Tyr Trp Gly Gln		
20	125	130	135
	Gly Thr Leu Val Thr Val Ser Ala Ala Ser Thr Lys Gly Pro Ser		
	140	145	150
	Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr		
25	155	160	165
	Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val		
	170	175	180
30	Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr		
	185	190	195
	Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser		
35	200	205	210
	Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile		
	215	220	225
	Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys		
40	230	235	240
	Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr		
	245	250 251	

45 (2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 22 base pairs

(B) TYPE: Nucleic Acid

50 (C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

55

CAGTCCAACT GTTCAGGACG CC 22

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH: 22 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

10 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

GTGCTGCTCA TGCTGTAGGT GC 22

15 (2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:

- 20 (A) LENGTH: 23 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

25

GAAGTTGATG TCTTGTGAGT GGC 23

(2) INFORMATION FOR SEQ ID NO:34:

30 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 24 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

35

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

GCATCCTAGA GTCACCGAGG AGCC 24

40

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:

- 45 (A) LENGTH: 22 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

50

CACTGGCTCA GGGAAATAAC CC 22

(2) INFORMATION FOR SEQ ID NO:36:

55

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 22 base pairs
- (B) TYPE: Nucleic Acid
- (C) STRANDEDNESS: Single
- (D) TOPOLOGY: Linear

5

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

GGAGAGCTGG GAAGGTGTGC AC 22

10

(2) INFORMATION FOR SEQ ID NO:37:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 37 base pairs
 - (B) TYPE: Nucleic Acid
 - (C) STRANDEDNESS: Single
 - (D) TOPOLOGY: Linear

15

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

20

CCAATGCATA CGCTGACATC GTGATGACCC AGACCCC 37

(2) INFORMATION FOR SEQ ID NO:38:

25

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 37 base pairs
 - ~~(B) TYPE: Nucleic Acid~~
 - (C) STRANDEDNESS: Single
 - (D) TOPOLOGY: Linear

30

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

CCAATGCATA CGCTGATATT GTGATGACTC AGACTCC 37

35

(2) INFORMATION FOR SEQ ID NO:39:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 37 base pairs
 - (B) TYPE: Nucleic Acid
 - (C) STRANDEDNESS: Single
 - (D) TOPOLOGY: Linear

40

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

45

CCAATGCATA CGCTGACATC GTGATGACAC AGACACC 37

50

(2) INFORMATION FOR SEQ ID NO:40:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 35 base pairs
 - ~~(B) TYPE: Nucleic Acid~~
 - (C) STRANDEDNESS: Single
 - (D) TOPOLOGY: Linear

55

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

5 AGATGTCAAT TGCTCACTGG ATGGTGGGAA GATGG 35

(2) INFORMATION FOR SEQ ID NO:41:

10 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

15 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

CAAACGCGTA CGCTGAGATC CAGCTGCAGC AG 32

20 (2) INFORMATION FOR SEQ ID NO:42:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32 base pairs
(B) TYPE: Nucleic Acid
25 (C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

30

CAAACGCGTA CGCTGAGATT CAGCTCCAGC AG 32

(2) INFORMATION FOR SEQ ID NO:43:

35 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 39 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
40 (D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

45 CGATGGGCCC GGATAGACCG ATGGGGCTGT TGTTTTGGC 39

(2) INFORMATION FOR SEQ ID NO:44:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 39 base pairs
50 (B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

55

CGATGGGCCC GGATAGACTG ATGGGGCTGT TGT TTTGGC 39

(2) INFORMATION FOR SEQ ID NO:45:

- 5 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 39 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
10 (D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

CGATGGGCCC GGATAGACAG ATGGGGCTGT TGT TTTGGC 39

15

(2) INFORMATION FOR SEQ ID NO:46:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 39 base pairs
20 (B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Double
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:

25

CGATGGGCCC GGATAGACGG ATGGGGCTGT TGT TTTGGC 39

(2) INFORMATION FOR SEQ ID NO:47:

30

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 391 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Double
35 (D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:

40 GATATCGTGA TGACACAGAC ACCACTCTCC CTGCCTGTCA GTCTTGAGAGA 50
TCAGGCCTCC ATCTCTTGCA GATCTAGTCA GAGCCTTGTA CACGGTATTG 100
GAAACACCTA TTTACATTGG TACCTGCAGA AGCCAGGCCA GTCTCCAAAG 150
45 CTCCTGATCT ACAAAGTTTC CAACCGATTT TCTGGGGTCC CAGACAGGTT 200
CAGTGGCAGT GGATCAGGGA CAGATTTTAC ACTCAGGATC AGCAGAGTGG 250
50 AGGCTGAGGA TCTGGGACTT TATTTCTGCT CTCAAAGTAC ACATGTTCCG 300
CTCACGTTTCG GTGCTGGGAC CAAGCTGGAG CTGAAACGGG CTGATGCTGC 350

55

ACCAACTGTA TCCATCTTCC CACCATCCAG TGAGCAATTG A 391

(2) INFORMATION FOR SEQ ID NO:48:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 131 amino acids

(B) TYPE: Amino Acid

(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:

10 Asp Ile Val Met Thr Gln Thr Pro Leu Ser Leu Pro Val Ser Leu
 1 5 10 15
 Gly Asp Gln Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Val
 20 25 30
 15 His Gly Ile Gly Asn Thr Tyr Leu His Trp Tyr Leu Gln Lys Pro
 35 40 45
 Gly Gln Ser Pro Lys Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe
 50 55 60
 20 Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp
 65 70 75
 Phe Thr Leu Arg Ile Ser Arg Val Glu Ala Glu Asp Leu Gly Leu
 80 85 90
 Tyr Phe Cys Ser Gln Ser Thr His Val Pro Leu Thr Phe Gly Ala
 95 100 105
 30 Gly Thr Lys Leu Glu Leu Lys Arg Ala Asp Ala Ala Pro Thr Val
 110 115 120
 Ser Ile Phe Pro Pro Ser Ser Glu Gln Leu Lys
 125 130 131

(2) INFORMATION FOR SEQ ID NO:49:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 405 base pairs

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Double

(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:

45 GAGATTCAGC TGCAGCAGTC TGGACCTGAG CTGATGAAGC CTGGGGCTTC 50
 AGTGAAGATA TCCTGCAAGG CTTCTGGTTA TTCATTCAGT AGCCACTACA 100
 50 TGCACTGGGT GAAGCAGAGC CATGGAAAGA GCCTTGAGTG GATTGGCTAC 150
 ATTGATCCTT CCAATGGTGA AACTACTTAC AACCAGAAAT TCAAGGGCAA 200
 55 GGCCACATTG ACTGTAGACA CATCTTCCAG CACAGCCAAC GTGCATCTCA 250

GCAGCCTGAC ATCTGATGAC TCTGCAGTCT ATTTCTGTGC AAGAGGGGAC 300
 TATAGATACA ACGGCGACTG GTTTTTCGAT GTCTGGGGNG NAGGGACCAC 350
 5 GGTCACCGTC TCCTCCGCCA AAACCGACAG CCCCATCGGT CTATCCGGGC 400
 CCATC 405

(2) INFORMATION FOR SEQ ID NO:50:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 135 amino acids
 (B) TYPE: Amino Acid
 (D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:50:

Glu	Ile	Gln	Leu	Gln	Gln	Ser	Gly	Pro	Glu	Leu	Met	Lys	Pro	Gly	1	5	10	15
Ala	Ser	Val	Lys	Ile	Ser	Cys	Lys	Ala	Ser	Gly	Tyr	Ser	Phe	Ser	20	25	30	
Ser	His	Tyr	Met	His	Trp	Val	Lys	Gln	Ser	His	Gly	Lys	Ser	Leu	35	40	45	
Glu	Trp	Ile	Gly	Tyr	Ile	Asp	Pro	Ser	Asn	Gly	Glu	Thr	Thr	Tyr	50	55	60	
Asn	Gln	Lys	Phe	Lys	Gly	Lys	Ala	Thr	Leu	Thr	Val	Asp	Thr	Ser	65	70	75	
Ser	Ser	Thr	Ala	Asn	Val	His	Leu	Ser	Ser	Leu	Thr	Ser	Asp	Asp	80	85	90	
Ser	Ala	Val	Tyr	Phe	Cys	Ala	Arg	Gly	Asp	Tyr	Arg	Tyr	Asn	Gly	95	100	105	
Asp	Trp	Phe	Phe	Asp	Val	Trp	Gly	Xaa	Gly	Thr	Thr	Val	Thr	Val	110	115	120	
Ser	Ser	Ala	Lys	Thr	Asp	Ser	Pro	Ile	Gly	Leu	Ser	Gly	Pro	Ile	125	130	135	

(2) INFORMATION FOR SEQ ID NO:51:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 22 base pairs
 (B) TYPE: Nucleic Acid
 (C) STRANDEDNESS: Single
 (D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:51:

CTTGGTGGAG GCGGAGGAGA CG 22

(2) INFORMATION FOR SEQ ID NO:52:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 38 base pairs
- (B) TYPE: Nucleic Acid
- (C) STRANDEDNESS: Single
- (D) TOPOLOGY: Linear

10 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:52:

GAAACGGGCT GTTGCTGCAC CAACTGTATT CATCTTCC 38

15 (2) INFORMATION FOR SEQ ID NO:53:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 31 base pairs
- (B) TYPE: Nucleic Acid
- (C) STRANDEDNESS: Single
- (D) TOPOLOGY: Linear

20 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:53:

25 GTCACCGTCT CCTCCGCCTC CACCAAGGGC C 31

(2) INFORMATION FOR SEQ ID NO:54:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 22 base pairs
- (B) TYPE: Nucleic Acid
- (C) STRANDEDNESS: Single
- (D) TOPOLOGY: Linear

35 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:54:

40 CTTGGTGGAG GCGGAGGAGA CG 22

40 (2) INFORMATION FOR SEQ ID NO:55:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 729 base pairs
- (B) TYPE: Nucleic Acid
- (C) STRANDEDNESS: Double
- (D) TOPOLOGY: Linear

45 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:55:

50 ATGAAGAAGA ATATCGCATT TCTTCTTGCA TCTATGTTG TTTTTTCTAT 50
TGCTACAAAT GCATACGCTG ATATCGTGAT GACACAGACA CCACTCTCCC 100
55 TGCCTGTCAG TCTTGGAGAT CAGGCTCCA TCTCTGCAG ATCTAGTCAG 150

AGCCTTGATAC ACGGTATTGG AAACACCTAT TTACATTGGT ACCTGCAGAA 200
 GCCAGGCCAG TCTCCAAAGC TCCTGATCTA CAAAGTTTCC AACCGATTTT 250
 5 CTGGGGTCCC AGACAGGTTC AGTGGCAGTG GATCAGGGAC AGATTTTACA 300
 CTCAGGATCA GCAGAGTGGA GGCTGAGGAT CTGGGACTTT ATTTCTGCTC 350
 10 TCAAAGTACA CATGTTCCGC TCACGTTCCG TGCTGGGACC AAGCTGGAGC 400
 TGAAACGGGC TGTGTGCTGCA CCAACTGTAT TCATCTTCCC ACCATCCAGT 450
 GAGCAATTGA AATCTGGAAC TGCCTCTGTT GTGTGCCTGC TGAATAACTT 500
 15 CTATCCCAGA GAGGCCAAAG TACAGTGGA GGTGGATAAC GCCCTCCAAT 550
 CGGGTAACTC CCAGGAGAGT GTCACAGAGC AGGACAGCAA GGACAGCACC 600
 20 TACAGCCTCA GCAGCACCCT GACGCTGAGC AAAGCAGACT ACGAGAAACA 650
 CAAAGTCTAC GCCTGCGAAG TCACCCATCA GGGCCTGAGC TCGCCCGTCA 700
 CAAAGAGCTT CAACAGGGGA GAGTGTTAA 729

25

(2) INFORMATION FOR SEQ ID NO:56:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 242 amino acids
 30 (B) TYPE: Amino Acid
 (D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:56:

35 Met Lys Lys Asn Ile Ala Phe Leu Leu Ala Ser Met Phe Val Phe
 1 5 10 15
 Ser Ile Ala Thr Asn Ala Tyr Ala Asp Ile Val Met Thr Gln Thr
 20 25 30
 40 Pro Leu Ser Leu Pro Val Ser Leu Gly Asp Gln Ala Ser Ile Ser
 35 40 45
 45 Cys Arg Ser Ser Gln Ser Leu Val His Gly Ile Gly Asn Thr Tyr
 50 55 60
 Leu His Trp Tyr Leu Gln Lys Pro Gly Gln Ser Pro Lys Leu Leu
 65 70 75
 50 Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro Asp Arg Phe
 80 85 90
 Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Arg Ile Ser Arg
 95 100 105
 55 Val Glu Ala Glu Asp Leu Gly Leu Tyr Phe Cys Ser Gln Ser Thr

	110	115	120
	His Val Pro Leu Thr Phe Gly Ala Gly	Thr Lys Leu Glu Leu Lys	
	125	130	135
5	Arg Ala Val Ala Ala Pro Thr Val Phe	Ile Phe Pro Pro Ser Ser	
	140	145	150
	Glu Gln Leu Lys Ser Gly Thr Ala Ser	Val Val Cys Leu Leu Asn	
10	155	160	165
	Asn Phe Tyr Pro Arg Glu Ala Lys Val	Gln Trp Lys Val Asp Asn	
	170	175	180
15	Ala Leu Gln Ser Gly Asn Ser Gln Glu	Ser Val Thr Glu Gln Asp	
	185	190	195
	Ser Lys Asp Ser Thr Tyr Ser Leu Ser	Ser Thr Leu Thr Leu Ser	
20	200	205	210
	Lys Ala Asp Tyr Glu Lys His Lys Val	Tyr Ala Cys Glu Val Thr	
	215	220	225
	His Gln Gly Leu Ser Ser Pro Val Thr	Lys Ser Phe Asn Arg Gly	
25	230	235	240
	Glu Cys		
	242		

30 (2) INFORMATION FOR SEQ ID NO:57:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 762 base pairs
 - (B) TYPE: Nucleic Acid
 - (C) STRANDEDNESS: Double
 - (D) TOPOLOGY: Linear

35

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:57:

40

ATGAAAAAGA ATATCGCATT TCTTCTTGCA TCTATGTTTCG TTTTTTCTAT 50

TGCTACAAAC GCGTACGCTG AGATTGAGCT GCAGCAGTCT GGACCTGAGC 100

45

TGATGAAGCC TGGGGCTTCA GTGAAGATAT CCTGCAAGGC TTCTGGTTAT 150

TCATTGAGTA GCCACTACAT GCACTGGGTG AAGCAGAGCC ATGGAAAGAG 200

50

CCTTGAGTGG ATTGGCTACA TTGATCCTTC CAATGGTGAA ACTACTTACA 250

ACCAGAAATT CAAGGGCAAG GCCACATTGA CTGTAGACAC ATCTTCCAGC 300

ACAGCCAACG TGCATCTCAG CAGCCTGACA TCTGATGACT CTGCAGTCTA 350

55

TTTCTGTGCA AGAGGGGACT ATAGATACAA CGGCGACTGG TTTTTCGATG 400

TCTGGGGCGC AGGGACCACG GTCACCGTCT CCTCCGCCTC CACCAAGGGC 450
 CCATCGGTCT TCCCCCTGGC ACCCTCCTCC AAGAGCACCT CTGGGGGCAC 500
 5 AGCGGCCCTG GGCTGCCTGG TCAAGGACTA CTTCCCCGAA CCGGTGACGG 550
 TGTCGTGGAA CTCAGGCGCC CTGACCAGCG GCGTGACAC CTTCCCGGCT 600
 GTCCTACAGT CCTCAGGACT CTACTCCCTC AGCAGCGTGG TGACCGTGCC 650
 10 CTCCAGCAGC TTGGGCACCC AGACCTACAT CTGCAACGTG AATCACAAGC 700
 CCAGCAACAC CAAGGTGGAC AAGAAAGTTG AGCCCAAATC TTGTGACAAA 750
 15 ACTCACACAT GA 762

(2) INFORMATION FOR SEQ ID NO:58:

(i) SEQUENCE CHARACTERISTICS:

- 20 (A) LENGTH: 253 amino acids
 (B) TYPE: Amino Acid
 (D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:58:

25 Met Lys Lys Asn Ile Ala Phe Leu Leu Ala Ser Met Phe Val Phe
 1 5 10 15
 Ser Ile Ala Thr Asn Ala Tyr Ala Glu Ile Gln Leu Gln Gln Ser
 30 20 25 30
 Gly Pro Glu Leu Met Lys Pro Gly Ala Ser Val Lys Ile Ser Cys
 35 35 40 45
 Lys Ala Ser Gly Tyr Ser Phe Ser Ser His Tyr Met His Trp Val
 50 55 60
 Lys Gln Ser His Gly Lys Ser Leu Glu Trp Ile Gly Tyr Ile Asp
 40 65 70 75
 Pro Ser Asn Gly Glu Thr Thr Tyr Asn Gln Lys Phe Lys Gly Lys
 80 85 90
 Ala Thr Leu Thr Val Asp Thr Ser Ser Ser Thr Ala Asn Val His
 45 95 100 105
 Leu Ser Ser Leu Thr Ser Asp Asp Ser Ala Val Tyr Phe Cys Ala
 110 115 120
 50 Arg Gly Asp Tyr Arg Tyr Asn Gly Asp Trp Phe Phe Asp Val Trp
 125 130 135
 Gly Ala Gly Thr Thr Val Thr Val Ser Ser Ala Ser Thr Lys Gly
 140 145 150
 55 Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly

		155		160		165
	Gly Thr Ala Ala	Leu Gly Cys Leu Val	Lys Asp Tyr Phe Pro Glu			
		170	175		180	
5	Pro Val Thr Val	Ser Trp Asn Ser Gly	Ala Leu Thr Ser Gly Val			
		185	190		195	
	His Thr Phe Pro	Ala Val Leu Gln Ser	Ser Gly Leu Tyr Ser Leu			
10		200	205		210	
	Ser Ser Val Val	Thr Val Pro Ser Ser	Ser Leu Gly Thr Gln Thr			
		215	220		225	
15	Tyr Ile Cys Asn	Val Asn His Lys Pro	Ser Asn Thr Lys Val Asp			
		230	235		240	
	Lys Lys Val Glu	Pro Lys Ser Cys Asp	Lys Thr His Thr			
		245	250		253	

20

(2) INFORMATION FOR SEQ ID NO:59:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 114 amino acids
 (B) TYPE: Amino Acid
 (D) TOPOLOGY: Linear

25

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:59:

30	Asp Ile Val Met Thr Gln Thr Pro Leu Ser Leu Pro Val Ser Leu	
	1 5 10 15	
	Gly Asp Gln Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Val	
	20 25 30	
35	His Gly Ile Gly Asn Thr Tyr Leu His Trp Tyr Leu Gln Lys Pro	
	35 40 45	
	Gly Gln Ser Pro Lys Leu Leu Ile Tyr Tyr Lys Val Ser Asn Arg	
40	50 55 60	
	Phe Ser Gly Val Pro Asp Arg Phe Ser Asp Ser Gly Ser Gly Thr	
	65 70 75	
45	Asp Phe Thr Leu Arg Ile Ser Arg Val Glu Ala Glu Asp Leu Gly	
	80 85 90	
	Leu Tyr Phe Cys Ser Gln Ser Thr His Val Pro Leu Thr Phe Gly	
	95 100 105	
50	Ala Gly Thr Lys Leu Glu Leu Lys Arg	
	110 114	

50

(2) INFORMATION FOR SEQ ID NO:60:

55

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 114 amino acids
 (B) TYPE: Amino Acid
 (D) TOPOLOGY: Linear

5 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:60:

Asp	Ile	Gln	Met	Thr	Gln	Ser	Pro	Ser	Ser	Leu	Ser	Ala	Ser	Val	
1				5					10					15	
Gly	Asp	Arg	Val	Thr	Ile	Thr	Cys	Arg	Ser	Ser	Gln	Ser	Leu	Val	
				20					25					30	
His	Gly	Ile	Gly	Asn	Thr	Tyr	Leu	His	Trp	Tyr	Gln	Gln	Lys	Pro	
				35					40					45	
Gly	Lys	Ala	Pro	Lys	Leu	Leu	Ile	Tyr	Tyr	Lys	Val	Ser	Asn	Arg	
				50					55					60	
Phe	Ser	Gly	Val	Pro	Ser	Arg	Phe	Ser	Gly	Ser	Gly	Ser	Gly	Thr	
				65					70					75	
Asp	Phe	Thr	Leu	Thr	Ile	Ser	Ser	Leu	Gln	Pro	Glu	Asp	Phe	Ala	
				80					85					90	
Thr	Tyr	Tyr	Cys	Ser	Gln	Ser	Thr	His	Val	Pro	Leu	Thr	Phe	Gly	
				95					100					105	
Gln	Gly	Thr	Lys	Val	Glu	Ile	Lys	Arg							
				110				114							

(2) INFORMATION FOR SEQ ID NO:61:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 109 amino acids
 (B) TYPE: Amino Acid
 (D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:61:

Asp	Ile	Gln	Met	Thr	Gln	Ser	Pro	Ser	Ser	Leu	Ser	Ala	Ser	Val	
1				5					10					15	
Gly	Asp	Arg	Val	Thr	Ile	Thr	Cys	Arg	Ala	Ser	Lys	Thr	Ile	Ser	
				20					25					30	
Lys	Tyr	Leu	Ala	Trp	Tyr	Gln	Gln	Lys	Pro	Gly	Lys	Ala	Pro	Lys	
				35					40					45	
Leu	Leu	Ile	Tyr	Tyr	Ser	Gly	Ser	Thr	Leu	Glu	Ser	Gly	Val	Pro	
				50					55					60	
Ser	Arg	Phe	Ser	Gly	Ser	Gly	Ser	Gly	Thr	Asp	Phe	Thr	Leu	Thr	
				65					70					75	
Ile	Ser	Ser	Leu	Gln	Pro	Glu	Asp	Phe	Ala	Thr	Tyr	Tyr	Cys	Gln	
				80					85					90	

Gln His Asn Glu Tyr Pro Leu Thr Phe Gly Gln Gly Thr Lys Val
 95 100 105

5 Glu Ile Lys Arg
 109

(2) INFORMATION FOR SEQ ID NO:62:

10 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 117 amino acids
 (B) TYPE: Amino Acid
 (D) TOPOLOGY: Linear

15 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:62:

Glu Ile Gln Leu Gln Gln Ser Gly Pro Glu Leu Met Lys Pro Gly
 1 5 10 15

20 Ala Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Ser Phe Ser
 20 25 30

Ser His Tyr Met His Trp Val Lys Gln Ser His Gly Lys Ser Leu
 35 40 45

25 Glu Trp Ile Gly Tyr Ile Asp Pro Ser Asn Gly Glu Thr Thr Tyr
 50 55 60

Asn Gln Lys Phe Lys Gly Lys Ala Thr Leu Thr Val Asp Thr Ser
 30 65 70 75

Ser Ser Thr Ala Asn Val His Leu Ser Ser Leu Thr Ser Asp Asp
 80 85 90

35 Ser Ala Val Tyr Phe Cys Ala Ala Arg Gly Asp Tyr Arg Tyr Asn
 95 100 105

Gly Asp Trp Phe Phe Asp Val Trp Gly Ala Gly Thr
 110 115 117

40

(2) INFORMATION FOR SEQ ID NO:63:

45 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 117 amino acids
 (B) TYPE: Amino Acid
 (D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:63:

50 Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly
 1 5 10 15

Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Tyr Ser Phe Ser
 20 25 30

55 Ser His Tyr Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu

	35	40	45
	Glu Trp Val Gly Tyr Ile Asp Pro Ser Asn Gly Glu Thr Thr Tyr		
	50	55	60
5	Asn Gln Lys Phe Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser		
	65	70	75
	Lys Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp		
10	80	85	90
	Thr Ala Val Tyr Tyr Cys Ala Ala Arg Gly Asp Tyr Arg Tyr Asn		
	95	100	105
15	Gly Asp Trp Phe Phe Asp Val Trp Gly Gln Gly Thr		
	110	115	117

(2) INFORMATION FOR SEQ ID NO:64:

- 20 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 116 amino acids
 (B) TYPE: Amino Acid
 (D) TOPOLOGY: Linear

- 25 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:64:

	Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly		
	1	5	10
30	Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Ser Phe Thr		
	20	25	30
	Gly His Trp Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu		
	35	40	45
35	Glu Trp Val Gly Met Ile His Pro Ser Asp Ser Glu Thr Arg Tyr		
	50	55	60
	Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser		
40	65	70	75
	Lys Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp		
	80	85	90
45	Thr Ala Val Tyr Tyr Cys Ala Ala Arg Gly Ile Tyr Phe Tyr Gly		
	95	100	105
	Thr Thr Tyr Phe Asp Tyr Trp Gly Gln Gly Thr		
50	110	115	116

(2) INFORMATION FOR SEQ ID NO:65:

- 55 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 242-amino acids
 (B) TYPE: Amino Acid
 (D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:65:

5	Met	Lys	Lys	Asn	Ile	Ala	Phe	Leu	Leu	Ala	Ser	Met	Phe	Val	Phe	1	5	10	15
	Ser	Ile	Ala	Thr	Asn	Ala	Tyr	Ala	Asp	Ile	Gln	Met	Thr	Gln	Ser	20	25	30	
10	Pro	Ser	Ser	Leu	Ser	Ala	Ser	Val	Gly	Asp	Arg	Val	Thr	Ile	Thr	35	40	45	
	Cys	Arg	Ser	Ser	Gln	Ser	Leu	Val	His	Gly	Ile	Gly	Asn	Thr	Tyr	50	55	60	
15	Leu	His	Trp	Tyr	Gln	Gln	Lys	Pro	Gly	Lys	Ala	Pro	Lys	Leu	Leu	65	70	75	
	Ile	Tyr	Lys	Val	Ser	Asn	Arg	Phe	Ser	Gly	Val	Pro	Ser	Arg	Phe	80	85	90	
20	Ser	Gly	Ser	Gly	Ser	Gly	Thr	Asp	Phe	Thr	Leu	Thr	Ile	Ser	Ser	95	100	105	
	Leu	Gln	Pro	Glu	Asp	Phe	Ala	Thr	Tyr	Tyr	Cys	Ser	Gln	Ser	Thr	110	115	120	
25	His	Val	Pro	Leu	Thr	Phe	Gly	Gln	Gly	Thr	Lys	Val	Glu	Ile	Lys	125	130	135	
30	Arg	Thr	Val	Ala	Ala	Pro	Ser	Val	Phe	Ile	Phe	Pro	Pro	Ser	Asp	140	145	150	
	Glu	Gln	Leu	Lys	Ser	Gly	Thr	Ala	Ser	Val	Val	Cys	Leu	Leu	Asn	155	160	165	
35	Asn	Phe	Tyr	Pro	Arg	Glu	Ala	Lys	Val	Gln	Trp	Lys	Val	Asp	Asn	170	175	180	
	Ala	Leu	Gln	Ser	Gly	Asn	Ser	Gln	Glu	Ser	Val	Thr	Glu	Gln	Asp	185	190	195	
40	Ser	Lys	Asp	Ser	Thr	Tyr	Ser	Leu	Ser	Ser	Thr	Leu	Thr	Leu	Ser	200	205	210	
45	Lys	Ala	Asp	Tyr	Glu	Lys	His	Lys	Val	Tyr	Ala	Cys	Glu	Val	Thr	215	220	225	
	His	Gln	Gly	Leu	Ser	Ser	Pro	Val	Thr	Lys	Ser	Phe	Asn	Arg	Gly	230	235	240	
50	Glu	Cys														242			

55 (2) INFORMATION FOR SEQ ID NO:66:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 253 amino acids

(B) TYPE: Amino Acid

(D) TOPOLOGY: Linear

5

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:66:

Met	Lys	Lys	Asn	Ile	Ala	Phe	Leu	Leu	Ala	Ser	Met	Phe	Val	Phe	1	5	10	15
Ser	Ile	Ala	Thr	Asn	Ala	Tyr	Ala	Glu	Val	Gln	Leu	Val	Gln	Ser	20	25	30	
Gly	Gly	Gly	Leu	Val	Gln	Pro	Gly	Gly	Ser	Leu	Arg	Leu	Ser	Cys	35	40	45	
Ala	Ala	Ser	Gly	Tyr	Ser	Phe	Ser	Ser	His	Tyr	Met	His	Trp	Val	50	55	60	
Arg	Gln	Ala	Pro	Gly	Lys	Gly	Leu	Glu	Trp	Val	Gly	Tyr	Ile	Asp	65	70	75	
Pro	Ser	Asn	Gly	Glu	Thr	Thr	Tyr	Asn	Gln	Lys	Phe	Lys	Gly	Arg	80	85	90	
Phe	Thr	Leu	Ser	Arg	Asp	Asn	Ser	Lys	Asn	Thr	Ala	Tyr	Leu	Gln	95	100	105	
Met	Asn	Ser	Leu	Arg	Ala	Glu	Asp	Thr	Ala	Val	Tyr	Tyr	Cys	Ala	110	115	120	
Arg	Gly	Asp	Tyr	Arg	Tyr	Asn	Gly	Asp	Trp	Phe	Phe	Asp	Val	Trp	125	130	135	
Gly	Gln	Gly	Thr	Leu	Val	Thr	Val	Ser	Ser	Ala	Ser	Thr	Lys	Gly	140	145	150	
Pro	Ser	Val	Phe	Pro	Leu	Ala	Pro	Ser	Ser	Lys	Ser	Thr	Ser	Gly	155	160	165	
Gly	Thr	Ala	Ala	Leu	Gly	Cys	Leu	Val	Lys	Asp	Tyr	Phe	Pro	Glu	170	175	180	
Pro	Val	Thr	Val	Ser	Trp	Asn	Ser	Gly	Ala	Leu	Thr	Ser	Gly	Val	185	190	195	
His	Thr	Phe	Pro	Ala	Val	Leu	Gln	Ser	Ser	Gly	Leu	Tyr	Ser	Leu	200	205	210	
Ser	Ser	Val	Val	Thr	Val	Pro	Ser	Ser	Ser	Leu	Gly	Thr	Gln	Thr	215	220	225	
Tyr	Ile	Cys	Asn	Val	Asn	His	Lys	Pro	Ser	Asn	Thr	Lys	Val	Asp	230	235	240	
Lys	Lys	Val	Glu	Pro	Lys	Ser	Cys	Asp	Lys	Thr	His	Thr						

55

245

250

253

(2) INFORMATION FOR SEQ ID NO:67:

- 5 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 159 amino acids
 (B) TYPE: Amino Acid
 (D) TOPOLOGY: Linear

10 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:67:

Ser Gly Gly Gly Ser Gly Ser Gly Asp Phe Asp Tyr Glu Lys Met
 1 5 10 15
 15 Ala Asn Ala Asn Lys Gly Ala Met Thr Glu Asn Ala Asp Glu Asn
 20 25 30
 Ala Leu Gln Ser Asp Ala Lys Gly Lys Leu Asp Ser Val Ala Thr
 35 40 45
 20 Asp Tyr Gly Ala Ala Ile Asp Gly Phe Ile Gly Asp Val Ser Gly
 50 55 60
 Leu Ala Asn Gly Asn Gly Ala Thr Gly Asp Phe Ala Gly Ser Ser
 25 65 70 75
 Asn Ser Gln Met Ala Gln Val Gly Asp Gly Asp Asn Ser Pro Leu
 80 85 90
 30 Met Asn Asn Phe Arg Gln Tyr Leu Pro Ser Leu Pro Gln Ser Val
 95 100 105
 Glu Cys Arg Pro Phe Val Phe Ser Ala Gly Lys Pro Tyr Glu Phe
 110 115 120
 35 Ser Ile Asp Cys Asp Lys Ile Asn Leu Phe Arg Gly Val Phe Ala
 125 130 135
 Phe Leu Leu Tyr Val Ala Thr Phe Met Tyr Val Phe Ser Thr Phe
 40 140 145 150
 Ala Asn Ile Leu Arg Asn Lys Glu Ser
 155 159

45 (2) INFORMATION FOR SEQ ID NO:68:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 780 base pairs
 (B) TYPE: Nucleic Acid
 50 (C) STRANDEDNESS: Single
 (D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:68:

55 ATGAAAAAGA ATATCGCATT TCTTCTTGCA TCTATGTTTCG TTTTCTCTAT 50

TGCTACAAAC GCATACGCTG ATATCCAGAT GACCCAGTCC CCGAGCTCCC 100
 TGTCCGCCTC TGTGGGCGAT AGGGTCACCA TCACCTGCAG GTCAAGTCAA 150
 5 AGCTTAGTAC ATGGTATAGG TAACACGTAT TTACTGTTT ATCAACAGAA 200
 ACCAGGAAAA GCTCCGAAAC TACTGATTTA CAAAGTATCC AATCGATTCT 250
 10 CTGGAGTCCC TTCTCGCTTC TCTGGATCCG GTTCTGGGAC GGATTTCACT 300
 CTGACCATCA GCAGTCTGCA GCCAGAAGAC TTCGCAACTT ATTACTGTTC 350
 ACAGAGTACT CATGTCCCGC TCACGTTTGG ACAGGGTACC AAGGTGGAGA 400
 15 TCAAACGAAC TGTGGCTGCA CCATCTGTCT TCATCTTCCC GCCATCTGAT 450
 GAGCAGTTGA AATCTGGAAC TGCTTCTGTT GTGTGCCTGC TGAATAACTT 500
 20 CTATCCCAGA GAGGCCAAAG TACAGTGGAA GGTGGATAAC GCCCTCCAAT 550
 CGGGTAACTC CCAGGAGAGT GTCACAGAGC AGGACAGCAA GGACAGCACC 600
 TACAGCCTCA GCAGCACCTT GACGCTGAGC AAAGCAGACT ACGAGAAACA 650
 25 CAAAGTCTAC GCCTGCGAAG TCACCCATCA GGGCCTGAGC TCGCCCGTCA 700
~~CAAAGAGCTT CAACAGGGGA GAGTGTAAAG CTGATGCTCT ACGCCCGACG 750~~
 30 CATCGTGGCC CTAGTACGCA ACTAGTCGTA 780

(2) INFORMATION FOR SEQ ID NO:69:

(i) SEQUENCE CHARACTERISTICS:
 35 (A) LENGTH: 242 amino acids
 (B) TYPE: Amino Acid
 (D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:69:

40 Met Lys Lys Asn Ile Ala Phe Leu Leu Ala Ser Met Phe Val Phe
 1 5 10 15
 Ser Ile Ala Thr Asn Ala Tyr Ala Asp Ile Gln Met Thr Gln Ser
 45 20 25 30
 Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr
 35 40 45
 50 Cys Arg Ser Ser Gln Ser Leu Val His Gly Ile Gly Asn Thr Tyr
 50 55 60
 Leu His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu
 -----65 70 75
 55 Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro Ser Arg Phe

	80	85	90
	Ser Gly Ser Gly Ser Gly Thr Asp Phe	Thr Leu Thr Ile Ser Ser	
	95	100	105
5	Leu Gln Pro Glu Asp Phe Ala Thr Tyr	Tyr Cys Ser Gln Ser Thr	
	110	115	120
	His Val Pro Leu Thr Phe Gly Gln Gly	Thr Lys Val Glu Ile Lys	
10	125	130	135
	Arg Thr Val Ala Ala Pro Ser Val Phe	Ile Phe Pro Pro Ser Asp	
	140	145	150
15	Glu Gln Leu Lys Ser Gly Thr Ala Ser	Val Val Cys Leu Leu Asn	
	155	160	165
	Asn Phe Tyr Pro Arg Glu Ala Lys Val	Gln Trp Lys Val Asp Asn	
	170	175	180
20	Ala Leu Gln Ser Gly Asn Ser Gln Glu	Ser Val Thr Glu Gln Asp	
	185	190	195
	Ser Lys Asp Ser Thr Tyr Ser Leu Ser	Ser Thr Leu Thr Leu Ser	
25	200	205	210
	Lys Ala Asp Tyr Glu Lys His Lys Val	Tyr Ala Cys Glu Val Thr	
	215	220	225
30	His Gln Gly Leu Ser Ser Pro Val Thr	Lys Ser Phe Asn Arg Gly	
	230	235	240
	Glu Cys		
	242		

35

(2) INFORMATION FOR SEQ ID NO:70:

(i) SEQUENCE CHARACTERISTICS:

40 (A) LENGTH: 253 amino acids
(B) TYPE: Amino Acid
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:70:

45	Met Lys Lys Asn Ile Ala Phe Leu Leu Ala Ser Met Phe Val Phe	
	1 5 10	15
	Ser Ile Ala Thr Asn Ala Tyr Ala Glu Val Gln Leu Val Glu Ser	
	20 25	30
50	Gly Gly Gly Leu Val Gln Pro Gly Gly Ser Leu Arg Leu Ser Cys	
	35 40	45
	Ala Ala Ser Gly Tyr Ser Phe Ser Ser His Tyr Met His Trp Val	
55	50 55	60

	Lys	Gln	Ala	Pro	Gly	Lys	Gly	Leu	Glu	Trp	Val	Gly	Tyr	Ile	Asp	
					65					70					75	
5	Pro	Ser	Asn	Gly	Glu	Thr	Thr	Tyr	Asn	Gln	Lys	Phe	Lys	Gly	Arg	
					80					85					90	
	Phe	Thr	Leu	Ser	Arg	Asp	Asn	Ser	Lys	Asn	Thr	Ala	Tyr	Leu	Gln	
					95					100					105	
10	Met	Asn	Ser	Leu	Arg	Ala	Glu	Asp	Thr	Ala	Val	Tyr	Tyr	Cys	Ala	
					110					115					120	
	Arg	Gly	Asp	Tyr	Arg	Tyr	Asn	Gly	Asp	Trp	Phe	Phe	Asp	Val	Trp	
					125					130					135	
15	Gly	Gln	Gly	Thr	Leu	Val	Thr	Val	Ser	Ser	Ala	Ser	Thr	Lys	Gly	
					140					145					150	
	Pro	Ser	Val	Phe	Pro	Leu	Ala	Pro	Ser	Ser	Lys	Ser	Thr	Ser	Gly	
20					155					160					165	
	Gly	Thr	Ala	Ala	Leu	Gly	Cys	Leu	Val	Lys	Asp	Tyr	Phe	Pro	Glu	
					170					175					180	
25	Pro	Val	Thr	Val	Ser	Trp	Asn	Ser	Gly	Ala	Leu	Thr	Ser	Gly	Val	
					185					190					195	
	His	Thr	Phe	Pro	Ala	Val	Leu	Gln	Ser	Ser	Gly	Leu	Tyr	Ser	Leu	
					200					205					210	
30	Ser	Ser	Val	Val	Thr	Val	Pro	Ser	Ser	Ser	Leu	Gly	Thr	Gln	Thr	
					215					220					225	
	Tyr	Ile	Cys	Asn	Val	Asn	His	Lys	Pro	Ser	Asn	Thr	Lys	Val	Asp	
35					230					235					240	
	Lys	Lys	Val	Glu	Pro	Lys	Ser	Cys	Asp	Lys	Thr	His	Thr			
					245					250			253			

40 (2) INFORMATION FOR SEQ ID NO:71:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 242 amino acids

(B) TYPE: Amino Acid

45 (D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:71:

50	Met	Lys	Lys	Asn	Ile	Ala	Phe	Leu	Leu	Ala	Ser	Met	Phe	Val	Phe	
	1				5					10					15	
	Ser	Ile	Ala	Thr	Asn	Ala	Tyr	Ala	Asp	Ile	Gln	Met	Thr	Gln	Ser	
					20					25					30	
55	Pro	Ser	Ser	Leu	Ser	Ala	Ser	Val	Gly	Asp	Arg	Val	Thr	Ile	Thr	
					35					40					45	

Cys Arg Ser Ser Gln Ser Leu Val His Gly Ile Gly Ala Thr Tyr
 50 55 60
 5 Leu His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu
 65 70 75
 Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro Ser Arg Phe
 80 85 90
 10 Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser
 95 100 105
 15 Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Ser Gln Ser Thr
 110 115 120
 His Val Pro Leu Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
 125 130 135
 20 Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp
 140 145 150
 Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn
 155 160 165
 25 Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn
 170 175 180
 30 Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp
 185 190 195
 Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser
 200 205 210
 35 Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr
 215 220 225
 His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly
 230 235 240
 40 Glu Cys
 242

(2) INFORMATION FOR SEQ ID NO:72:

45

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 45 amino acids
 (B) TYPE: Amino Acid
 (D) TOPOLOGY: Linear

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:72:

Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Arg Met Lys
 1 5 10 15
 55 Gln Leu Glu Asp Lys Val Glu Glu Leu Leu Ser Lys Asn Tyr His

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Leu Glu Asn Glu Val Ala Arg Leu Lys Lys Leu Val Gly Glu Arg
 35 40 45

5

(2) INFORMATION FOR SEQ ID NO:73:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 780 base pairs
 (B) TYPE: Nucleic Acid
 (C) STRANDEDNESS: Single
 (D) TOPOLOGY: Linear

10

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:73:

15

ATGAAAAAGA ATATCGCATT TCTTCTTGCA TCTATGTTCG TTTTCTCTAT 50
 TGCTACAAAC GCATACGCTG ATATCCAGAT GACCCAGTCC CCGAGCTCCC 100
 TGTCCGCCTC TGTGGGCGAT AGGGTCACCA TCACCTGCAG GTCAAGTCAA 150
 AGCTTAGTAC ATGGTATAGG TGCTACGTAT TTACACTGGT ATCAACAGAA 200
 ACCAGGAAAA GCTCCGAAAC TACTGATTTA CAAAGTATCC AATCGATTCT 250
 CTGGAGTCCC TTCTCGCTTC TCTGGATCCG GTTCTGGGAC GGATTTCACT 300
 CTGACCATCA GCAGTCTGCA GCCAGAAGAC TTCGCAACTT ATTACTGTTC 350
 ACAGAGTACT CATGTCCCGC TCACGTTTGG ACAGGGTACC AAGGTGGAGA 400
 TCAAACGAAC TGTGGCTGCA CCATCTGTCT TCATCTTCCC GCCATCTGAT 450
 GAGCAGTTGA AATCTGGAAC TGCTTCTGTT GTGTGCCTGC TGAATAACTT 500
 CTATCCCAGA GAGGCCAAAG TACAGTGGAA GGTGGATAAC GCCCTCCAAT 550
 CGGGTAACTC CCAGGAGAGT GTCACAGAGC AGGACAGCAA GGACAGCACC 600
 TACAGCCTCA GCAGCACCTT GACGCTGAGC AAAGCAGACT ACGAGAAACA 650
 CAAAGTCTAC GCCTGCGAAG TCACCCATCA GGGCCTGAGC TCGCCCGTCA 700
 CAAAGAGCTT CAACAGGGGA GAGTGTTAAG CTGATCCTCT ACGCCGGACG 750
 CATCGTGGCC CTAGTACGCA ACTAGTCGTA 780

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(2) INFORMATION FOR SEQ ID NO:74:

50

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 927 base pairs
 (B) TYPE: Nucleic Acid
 (C) STRANDEDNESS: Single
 (D) TOPOLOGY: Linear

55

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:74:

5 AAAAGGGTAT CTAGAGGTTG AGGTGATTTT ATGAAAAAGA ATATCGCATT 50
 TCTTCTTGCA TCTATGTTTCG TTTTCTCTAT TGCTACAAAC GCGTACGCTG 100
 AGGTTTCAGCT AGTGCAGTCT GCGGGTGGCC TGGTGCAGCC AGGGGGCTCA 150
 10 CTCCGTTTGT CCTGTGCAGC TTCTGGCTAC TCCTTCTCGA GTCACTATAT 200
 GCACTGGGTC CGTCAGGCCC CGGGTAAGGG CCTGGAATGG GTTGGATATA 250
 TTGATCCTTC CAATGGTGAA ACTACGTATA ATCAAAAGTT CAAGGGCCGT 300
 15 TTCACTTTAT CTCGCGACAA CTCCAAAAAC ACAGCATACC TGCAGATGAA 350
 CAGCCTGCGT GCTGAGGACA CTGCCGTCTA TTA CTGTGCA AGAGGGGATT 400
 20 ATCGCTACAA TGGTGACTGG TTCTTCGACG TCTGGGGTCA AGGAACCCTG 450
 GTCACCGTCT CCTCGGCCTC CACCAAGGGC CCATCGGTCT TCCCCCTGGC 500
 ACCCTCCTCC AAGAGCACCT CTGGGGGCAC AGCGGCCCTG GGCTGCCTGG 550
 25 TCAAGGACTA CTTCCCCGAA CCGGTGACGG TGTCGTGGAA CTCAGGCGCC 600
 CTGACCAGCG GCGTGACAC CTTCCCGGCT GTCCTACAGT CCTCAGGACT 650
 30 CTACTCCCTC AGCAGCGTGG TGACCGTGCC CTCCAGCAGC TTGGGCACCC 700
 AGACCTACAT CTGCAACGTG AATCACAAGC CCAGCAACAC CAAGGTCGAC 750
 AAGAAAGTTG AGCCCCAATC TTGTGACAAA ACTCACACAT GCCCCCGGTG 800
 35 CCCAGCACCA GAACTGCTGG GCGGCCGCAT GAAACAGCTA GAGGACAAGG 850
 TCGAAGAGCT ACTCTCCAAG AACTACCACC TAGAGAATGA AGTGGCAAGA 900
 40 CTCAAAAAGC TTGTCGGGGA GCGCTAA 927

(2) INFORMATION FOR SEQ ID NO:75:

45 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 298 amino acids
 (B) TYPE: Amino Acid
 (D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:75:

50 Met Lys Lys Asn Ile Ala Phe Leu Leu Ala Ser Met Phe Val Phe
 1 5 10 15
 Ser Ile Ala Thr Asn Ala Tyr Ala Glu Val Gln Leu Val Gln Ser
 55 20 25 30

	Gly Gly Gly Leu Val Gln Pro Gly Gly Ser Leu Arg Leu Ser Cys	35	40	45
5	Ala Ala Ser Gly Tyr Ser Phe Ser Ser His Tyr Met His Trp Val	50	55	60
	Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Gly Tyr Ile Asp	65	70	75
10	Pro Ser Asn Gly Glu Thr Thr Tyr Asn Gln Lys Phe Lys Gly Arg	80	85	90
	Phe Thr Leu Ser Arg Asp Asn Ser Lys Asn Thr Ala Tyr Leu Gln	95	100	105
15	Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala	110	115	120
	Arg Gly Asp Tyr Arg Tyr Asn Gly Asp Trp Phe Phe Asp Val Trp	125	130	135
20	Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly	140	145	150
	Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly	155	160	165
	Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu	170	175	180
30	Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val	185	190	195
	His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu	200	205	210
35	Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr	215	220	225
	Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp	230	235	240
	Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro	245	250	255
45	Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Arg Met Lys Gln Leu	260	265	270
	Glu Asp Lys Val Glu Glu Leu Leu Ser Lys Asn Tyr His Leu Glu	275	280	285
50	Asn Glu Val Ala Arg Leu Lys Lys Leu Val Gly Glu Arg	290	295	298

55 (2) INFORMATION FOR SEQ ID NO:76:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 6563 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:76:

10 GAATTCAACT TCTCCATACT TTGGATAAGG AAATACAGAC ATGAAAAATC 50
TCATTGCTGA GTTGTATTATTT AAGCTTGCCC AAAAAGAAGA AGAGTCGAAT 100
GAACTGTGTG CGCAGGTAGA AGCTTTGGAG ATTATCGTCA CTGCAATGCT 150
15 TCGCAATATG GCGCAAATG ACCAACAGCG GTTGATTGAT CAGGTAGAGG 200
GGGCGCTGTA CGAGGTAAAG CCCGATGCCA GCATTCCTGA CGACGATACG 250
20 GAGCTGCTGC GCGATTACGT AAAGAAGTTA TTGAAGCATC CTCGTAGTA 300
AAAAGTTAAT CTTTTCAACA GCTGTCATAA AGTTGTCACG GCCGAGACTT 350
ATAGTCGCTT TGTTTTTATT TTTTAATGTA TTTGTAATA GAATTCGAGC 400
25 TCGGTACCCG GGGATCCTCT CGAGGTTGAG GTGATTTTAT GAAAAAGAAT 450
ATCGCATTTC TTCTTGATC TATGTTTCGTT TTTTCTATTG CTACAAACGC 500
30 ATACGCTGAT ATCCAGATGA CCCAGTCCCC GAGCTCCCTG TCCGCCTCTG 550
TGGGCGATAG GGTCAACATC ACCTGCAGGT CAAGTCAAAG CTTAGTACAT 600
GGTATAGGTG CTACGTATTT AACTGGTAT CAACAGAAAC CAGGAAAAGC 650
35 TCCGAAACTA CTGATTTACA AAGTATCCAA TCGATTCTCT GGAGTCCCTT 700
CTCGCTTCTC TGGATCCGGT TCTGGGACGG ATTTCACTCT GACCATCAGC 750
40 AGTCTGCAGC CAGAAGACTT CGCAACTTAT TACTGTTTAC AGAGTACTCA 800
TGTCCCGCTC ACGTTTGGAC AGGGTACCAA GGTGGAGATC AAACGAACTG 850
TGGCTGCACC ATCTGTCTTC ATCTTCCCGC CATCTGATGA GCAGTTGAAA 900
45 TCTGGAAGTG CTTCTGTTGT GTGCCTGCTG AATAACTTCT ATCCAGAGA 950
GGCCAAAGTA CAGTGAAGG TGGATAACGC CCTCCAATCG GGTAAGTCCC 1000
50 AGGAGAGTGT CACAGAGCAG GACAGCAAGG ACAGCACCTA CAGCCTCAGC 1050
AGCACCTGA CGCTGAGCAA AGCAGACTAC GAGAAACACA AAGTCTACGC 1100
CTGCGAAGTC ACCCATCAGG GCCTGAGCTC GCCCGTCACA AAGAGCTTCA 1150
55 ACAGGGGAGA GTGTTAAGCT GATCCTCTAC GCCGGACGCA TCGTGGCCCT 1200

AGTACGCAAC TAGTCGTAAA AAGGGTATCT AGAGGTTGAG GTGATTTTAT 1250
 5 GAAAAAGAAT ATCGCATTTC TTCTTGCATC TATGTTTCGTT TTTTCTATTG 1300
 CTACAAACGC GTACGCTGAG GTTCAGCTAG TGCAGTCTGG CCGTGGCCTG 1350
 GTGCAGCCAG GGGGCTCACT CCGTTTGTCC TGTGCAGCTT CTGGCTACTC 1400
 10 CTTCTCGAGT CACTATATGC ACTGGGTCCG TCAGGCCCCG GGTAAGGGCC 1450
 TGGAAATGGGT TGGATATATT GATCCTTCCA ATGGTGAAAC TACGTATAAT 1500
 CAAAAGTTCA AGGGCCGTTT CACTTTATCT CGCGACAAC CAAAAACAC 1550
 15 AGCATACCTG CAGATGAACA GCCTGCGTGC TGAGGACACT GCCGTCTATT 1600
 ACTGTGCAAG AGGGGATTAT CGCTACAATG GTGACTGGTT CTTCGACGTC 1650
 20 TGGGGTCAAG GAACCCTGGT CACCGTCTCC TCGGCCTCCA CCAAGGGCCC 1700
 ATCGGTCTTC CCCCTGGCAC CCTCCTCCAA GAGCACCTCT GGGGGCACAG 1750
 CGGCCCTGGG CTGCCTGGTC AAGGACTACT TCCCCGAACC GGTGACGGTG 1800
 25 TCGTGGAAC CAGGCGCCCT GACCAGCGGC GTGCACACCT TCCCGGCTGT 1850
~~CCTACAGTCC TCAGGACTCT ACTCCCTCAG CAGCGTGGTG ACCGTGCCCT 1900~~
 30 CCAGCAGCTT GGGCACCCAG ACCTACATCT GCAACGTGAA TCACAAGCCC 1950
 AGCAACACCA AGGTCGACAA GAAAGTTGAG CCCAAATCTT GTGACAAAAC 2000
 TCACACATGC CCGCCGTGCC CAGCACCAGA ACTGCTGGGC GGCCGCATGA 2050
 35 AACAGCTAGA GGACAAGGTC GAAGAGCTAC TCTCCAAGAA CTACCACCTA 2100
 GAGAATGAAG TGGCAAGACT CAAAAGCTT GTCGGGGAGC GCTAAGCATG 2150
 40 CGACGGCCCT AGAGTCCCTA ACGCTCGGTT GCCGCCGGGC GTTTTTTATT 2200
 GTTAACTCAT GTTTGACAGC TTATCATCGA TAAGCTTTAA TGCGGTAGTT 2250
 TATCACAGTT AAATTGCTAA CGCAGTCAGG CACCGTGTAT GAAATCTAAC 2300
 45 AATGCGCTCA TCGTCATCCT CGGCACCGTC ACCCTGGATG CTGTAGGCAT 2350
 AGGCTTGGTT ATGCCGGTAC TGCCGGGCCT CTGCGGGAT ATCGTCCATT 2400
 50 CCGACAGCAT CGCCAGTCAC TATGGCGTGC TGCTAGCGCT ATATGCGTTG 2450
 ATGCAATTTT TATGCGCACC CGTTCTCGGA GCACTGTCCG ACCGCTTTGG 2500
~~CCGCCGCCCA GTCCTGCTCG CTTGCTACT TGGAGCCACT ATCGACTACG 2550~~
 55 CGATCATGGC GACCACACCC GTCCTGTGGA TCCTCTACGC CGGACGCATC 2600

GTGGCCGGCA TCACCGGCGC CACAGGTGCG GTTGCTGGCG CCTATATCGC 2650
CGACATCACC GATGGGGAAG ATCGGGCTCG CCACTTCGGG CTCATGAGCG 2700
5 CTTGTTTTCG CGTGGGTATG GTGGCAGGCC CCGTGGCCGG GGGACTGTTG 2750
GGCGCCATCT CCTTGACGC ACCATTCTT GCGGCGGCGG TGCTCAACGG 2800
10 CCTCAACCTA CTACTGGGCT GCTTCCTAAT GCAGGAGTCG CATAAGGGAG 2850
AGCGTCGTCC GATGCCCTTG AGAGCCTTCA ACCCAGTCAG CTCCTTCCGG 2900
TGCGCGCGGG GCATGACTAT CGTCGCCGCA CTTATGACTG TCTTCTTTAT 2950
15 CATGCAACTC GTAGGACAGG TGCCGGCAGC GCTCTGGGTC ATTTTCGGCG 3000
AGGACCCTT TCGCTGGAGC GCGACGATGA TCGGCCTGTC GCTTGCGGTA 3050
20 TTCGGAATCT TGCACGCCCT CGCTCAAGCC TTCGTACTG GTCCCGCCAC 3100
CAAACGTTTC GCGGAGAAGC AGGCCATTAT CGCCGGCATG GCGGCCGACG 3150
CGCTGGGCTA CGTCTTGCTG GCGTTCGCGA CGCGAGGCTG GATGGCCTTC 3200
25 CCCATTATGA TTCTTCTCGC TTCCGGCGGC ATCGGGATGC CCGCGTTGCA 3250
GGCCATGCTG TCCAGGCAGG TAGATGACGA CCATCAGGGA CAGCTTCAAG 3300
30 GATCGCTCGC GGCTCTTACC AGCCTAAGTT CGATCACTGG ACCGCTGATC 3350
GTCACGGCGA TTTATGCCGC CTCGGCGAGC ACATGGAACG GGTGCGCATG 3400
GATTGTAGGC GCCGCCCTAT ACCTTGTCTG CCTCCCCGCG TTGCGTCGCG 3450
35 GTGCATGGAG CCGGGCCACC TCGACCTGAA TGAAGCCGG CGGCACCTCG 3500
CTAACGGATT CACCACTCCA AGAATTGGAG CCAATCAATT CTTGCGGAGA 3550
40 ACTGTGAATG CGCAAACCAA CCCTTGGCAG AACATATCCA TCGCGTCCGC 3600
CATCTCCAGC AGCCGCACGC GGCGCATCTC GGGCAGCGTT GGGTCCTGGC 3650
CACGGGTGCG CATGATCGTG CTCCTGTCGT TGAGGACCCG GCTAGGCTGG 3700
45 CGGGGTGCGG TTAAGGTTA GCAGAATGAA TCACCGATAC GCGAGCGAAC 3750
GTGAAGCGAC TGCTGCTGCA AAACGTCTGC GACCTGAGCA ACAACATGAA 3800
50 TGGTCTTCGG TTTCCGTGTT TCGTAAAGTC TGGAAACGCG GAAGTCAGCG 3850
CCCTGCACCA TTATGTTCCG GATCTGCATC GCAGGATGCT GCTGGCTACC 3900
CTGTGGAACA CCTACATCTG TATTAACGAA GCGCTGGCAT TGACCCTGAG 3950
55 TGATTTTTCT CTGGTCCCGC CGCATCCATA CCGCCAGTTG TTTACCCTCA 4000

CAACGTTCCA GTAACCGGGC ATGTTTCATCA TCAGTAACCC GTATCGTGAG 4050
CATCCTCTCT CGTTTCATCG GTATCATTAC CCCCATGAAC AGAAATTCCC 4100
5 CCTTACACGG AGGCATCAAG TGACCAAACA GGAAAAAACC GCCCTTAACA 4150
TGGCCCGCTT TATCAGAAGC CAGACATTAA CGTTTCTGGA GAAACTCAAC 4200
10 GAGCTGGACG CGGATGAACA GGCAGACATC TGTGAATCGC TTCACGACCA 4250
CGCTGATGAG CTTTACCGCA GCTGCCTCGC GCGTTTCGGT GATGACGGTG 4300
AAAACCTCTG ACACATGCAG CTCCCGGAGA CGGTCACAGC TTGTCTGTAA 4350
15 GCGGATGCCG GGAGCAGACA AGCCCGTCAG GGC GCGTCAG CGGGTGTTGG 4400
CGGGTGTCGG GGC GAGCCA TGACCCAGTC ACGTAGCGAT AGCGGAGTGT 4450
20 ATACTGGCTT AACTATGCGG CATCAGAGCA GATTGTACTG AGAGTGCACC 4500
ATATGCGGTG TGAAATACCG CACAGATGCG TAAGGAGAAA ATACCGCATC 4550
AGGCGCTCTT CCGCTTCCTC GCTCACTGAC TCGCTGCGCT CGGTCGTTTCG 4600
25 GCTGCGGCGA GCGGTATCAG CTCACTCAAA GCGGTAATA CGGTTATCCA 4650
CAGAATCAGG GGATAACGCA GGAAAGAACA TGTGAGCAAA AGGCCAGCAA 4700
30 AAGGCCAGGA ACCGTAAAAA GGCCGCGTTG CTGGCGTTTT TCCATAGGCT 4750
CCGCCCCCCT GACGAGCATC ACAAAAATCG ACGCTCAAGT CAGAGGTGGC 4800
GAAACCCGAC AGGACTATAA AGATACCAGG CGTTTCCCCC TGGAAGCTCC 4850
35 CTCGTGCGCT CTCCTGTTCC GACCCTGCCG CTTACCGGAT ACCTGTCCGC 4900
CTTTCTCCCT TCGGGAAGCG TGGCGCTTTC TCATAGCTCA CGCTGTAGGT 4950
40 ATCTCAGTTC GGTGTAGGTC GTTCGCTCCA AGCTGGGCTG TGTGCACGAA 5000
CCCCCGTTC AGCCCGACCG CTGCGCCTTA TCCGTAACCT ATCGTCTTGA 5050
GTCCAACCCG GTAAGACACG ACTTATCGCC ACTGGCAGCA GCCACTGGTA 5100
45 ACAGGATTAG CAGAGCGAGG TATGTAGGCG GTGCTACAGA GTTCTTGAAG 5150
TGGTGGCCTA ACTACGGCTA CACTAGAAGG ACAGTATTTG GTATCTGCGC 5200
50 TCTGCTGAAG CCAGTTACCT TCGGAAAAAG AGTTGGTAGC TCTTGATCCG 5250
GCAAACAAAC CACCGCTGGT AGCGGTGGTT TTTTGTGTTG CAAGCAGCAG 5300
ATTACGCGCA GAAAAAAGG ATCTCAAGAA GATCCTTTGA TCTTTTCTAC 5350
55 GGGGTCTGAC GCTCAGTGGA ACGAAAATC ACGTTAAGGG ATTTTGGTCA 5400

TGAGATTATC AAAAAGGATC TTCACCTAGA TCCTTTTAAA TTAAAAATGA 5450
AGTTTTAAAT CAATCTAAAG TATATATGAG TAAACTTGGT CTGACAGTTA 5500
5 CCAATGCTTA ATCAGTGAGG CACCTATCTC AGCGATCTGT CTATTTCGTT 5550
CATCCATAGT TGCCTGACTC CCCGTCGTGT AGATAACTAC GATACGGGAG 5600
10 GGCTTACCAT CTGGCCCCAG TGCTGCAATG ATACCGCGAG ACCCACGCTC 5650
ACCGGCTCCA GATTTATCAG CAATAAACCA GCCAGCCGGA AGGGCCGAGC 5700
GCAGAAGTGG TCCTGCAACT TTATCCGCCT CCATCCAGTC TATTAATTGT 5750
15 TGCCGGGAAG CTAGAGTAAG TAGTTCGCCA GTTAATAGTT TGCGCAACGT 5800
TGTTGCCATT GCTGCAGGCA TCGTGGTGTC ACGCTCGTCG TTTGGTATGG 5850
20 CTTCAATCAG CTCCGGTTCC CAACGATCAA GGCGAGTTAC ATGATCCCCC 5900
ATGTTGTGCA AAAAAGCGGT TAGCTCCTTC GGTCTCCGA TCGTTGTCAG 5950
AAGTAAGTTG GCCGCAGTGT TATCACTCAT GGTTATGGCA GCACTGCATA 6000
25 ATTCTCTTAC TGTCATGCCA TCCGTAAGAT GCTTTTCTGT GACTGGTGAG 6050
TACTCAACCA AGTCATTCTG AGAATAGTGT ATGCGGCGAC CGAGTTGCTC 6100
30 TTGCCCCGGC TCAACACGGG ATAATACCGC GCCACATAGC AGAACTTTAA 6150
AAGTGCTCAT CATTGGAAAA CGTTCTTCGG GGCGAAAACCT CTCAAGGATC 6200
TTACCGCTGT TGAGATCCAG TTCGATGTAA CCCACTCGTG CACCCAACCTG 6250
35 ATCTTCAGCA TCTTTTACTT TCACCAGCGT TTCTGGGTGA GCAAAAACAG 6300
GAAGGCAAAA TGCCGCAAAA AAGGGAATAA GGGCGACACG GAAATGTTGA 6350
40 ATACTCATAC TCTTCCTTTT TCAATATTAT TGAAGCATTT ATCAGGGTTA 6400
TTGTCTCATG AGCGGATACA TATTTGAATG TATTTAGAAA AATAAACAAA 6450
TAGGGGTTCC GCGCACATTT CCCCAGAAAAG TGCCACCTGA CGTCTAAGAA 6500
45 ACCATTATTA TCATGACATT AACCTATAAA AATAGGCGTA TCACGAGGCC 6550
CTTTCGTCTT CAA 6563

WE CLAIM:

1. A conjugate consisting essentially of one or more antibody fragments covalently attached to one or more nonproteinaceous polymer molecules, wherein the apparent size of the conjugate is at least about 500 kD.
5
2. The conjugate of claim 1, wherein the apparent size of the conjugate is at least about 800 kD.
- 10 3. The conjugate of claim 1, wherein the apparent size of the conjugate is at least about 1,400 kD.
4. The conjugate of claim 1, wherein the apparent size of the conjugate is at least about 1,800 kD.
15
5. The conjugate of claim 1, wherein the apparent size of the conjugate is at least about 8 fold greater than the apparent size of the antibody fragment.
- ~~6. The conjugate of claim 5, wherein the apparent size of the conjugate is at least about 15~~
20 fold greater than the apparent size of the antibody fragment.
7. The conjugate of claim 6, wherein the apparent size of the conjugate is at least about 25 fold greater than the apparent size of the antibody fragment.
- 25 8. The conjugate of claim 1, wherein the conjugate contains no more than one antibody fragment, and wherein the antibody fragment is selected from the group consisting of Fab, Fab', Fab'-SH, Fv, scFv and F(ab')₂.
9. The conjugate of claim 8 wherein the antibody fragment is F(ab')₂.
30
10. The conjugate of claim 1 wherein the antibody fragment is covalently attached to no more than about 10 nonproteinaceous polymer molecules.
11. The conjugate of claim 10 wherein the antibody fragment is covalently attached to no
35 more than about 5 nonproteinaceous polymer molecules.

12. The conjugate of claim 11 wherein the antibody fragment is covalently attached to no more than about 2 nonproteinaceous polymer molecules.

13. The conjugate of claim 12 wherein the antibody fragment is attached to no more than 1 nonproteinaceous polymer molecule.

14. The conjugate of claim 12, wherein the antibody fragment comprises a heavy chain and a light chain derived from a parental antibody, wherein in the parental antibody the heavy and light chains are covalently linked by a disulfide bond between a cysteine residue in the light chain and a cysteine residue in the heavy chain, wherein in the antibody fragment the cysteine residue in the light or heavy chain is substituted with another amino acid and the cysteine residue in the opposite chain is covalently linked to a nonproteinaceous polymer molecule.

15. The conjugate of claim 8 wherein the antibody fragment is selected from the group consisting of Fab, Fab' and Fab'-SH.

16. The conjugate of claim 15 wherein the antibody fragment is covalently attached to no more than 1 nonproteinaceous polymer molecule.

17. The conjugate of claim 16 wherein the nonproteinaceous polymer molecule in the conjugate is covalently attached to the hinge region of the antibody fragment.

18. The conjugate of claim 1 wherein the nonproteinaceous polymer is a polyethylene glycol (PEG).

19. The conjugate of claim 18 wherein the PEG has an average molecular weight of at least about 20 kD.

20. The conjugate of claim 19 wherein the PEG has an average molecular weight of at least about 40 kD.

21. The conjugate of claim 20 wherein the PEG is a single chain molecule.

22. The conjugate of claim 20 wherein the PEG is a branched chain molecule.

23. The conjugate of claim 19, wherein the conjugate contains no more than one antibody fragment, and wherein the antibody fragment is a $F(ab')_2$ and is covalently attached to no more than about 2 PEG molecules.

5 24. The conjugate of claim 19, wherein the conjugate contains no more than one antibody fragment, and wherein the antibody fragment is selected from the group consisting of Fab, Fab' and Fab'-SH and is covalently attached to no more than one PEG molecule.

10 25. The conjugate of claim 24 wherein the PEG molecule is covalently attached to the hinge region of the antibody fragment.

26. The conjugate of claim 1 wherein the antibody fragment has an antigen binding site that binds to human IL-8.

15 27. The conjugate of claim 26, wherein the conjugate contains no more than one antibody fragment, wherein the antibody fragment is selected from the group consisting of Fab, Fab' and Fab'-SH, wherein the antibody fragment is covalently attached to no more than one nonproteinaceous polymer molecule, and wherein the nonproteinaceous polymer molecule is a polyethylene glycol having an actual molecular weight of at least about 30 kD.

20 28. The conjugate of claim 1 wherein the antibody fragment is humanized.

29. The conjugate of claim 1 wherein the conjugate contains no more than one antibody fragment.

25 30. A composition comprising the conjugate of claim 1 and a carrier.

31. The composition of claim 30 that is sterile.

30 32. A conjugate formed by one or more antibody fragments covalently attached to one or more nonproteinaceous polymer molecules, wherein the apparent size of the conjugate is at least about 500 kD, and wherein the molecular structure of the conjugate is free of other matter.

35 33. A conjugate formed by one or more antibody fragments covalently attached to one or more nonproteinaceous polymer molecules, wherein the apparent size of the conjugate is at least about 500 kD, wherein the antibody fragment incorporates a nonproteinaceous label free of any polymer, and wherein the molecular structure of the conjugate is free of other matter.

34. The conjugate of claim 33 wherein the nonproteinaceous label is a radiolabel.

35. A polypeptide selected from the group consisting of: (1) a polypeptide that is an anti-IL-8 monoclonal antibody or antibody fragment comprising a light chain amino acid sequence comprising the complementarity determining regions of the light chain polypeptide amino acid sequence of Fig. 36; and (2) a polypeptide that is an anti-IL-8 monoclonal antibody or antibody fragment comprising a light chain amino acid sequence comprising the complementarity determining regions of the light chain polypeptide amino acid sequence of Fig. 45.

10

36. The polypeptide of claim 35, wherein the light chain amino acid sequence comprises the complementarity determining regions of the light chain polypeptide amino acid sequence of Fig. 45.

37. The polypeptide of claim 35 that further comprises a heavy chain amino acid sequence comprising the complementarity determining regions of the heavy chain polypeptide amino acid sequence of Figs. 37A-37B.

38. The polypeptide of claim 35 wherein the light chain amino acid sequence is selected from the group consisting of: (1) a light chain amino acid sequence comprising amino acids 1-219 of the light chain polypeptide amino acid sequence of Fig. 36; and (2) a light chain amino acid sequence comprising amino acids 1-219 of the light chain polypeptide amino acid sequence of Fig. 45.

39. The polypeptide of claim 38 wherein the light chain amino acid sequence comprises amino acids 1-219 of the light chain amino acid sequence of Fig. 45.

25

40. The polypeptide of claim 38 that further comprises a heavy chain amino acid sequence comprising amino acids 1-230 of the heavy chain polypeptide amino acid sequence of Figs. 37A-37B.

41. The polypeptide of claim 40, wherein the heavy chain amino acid sequence is fused at its C-terminus to a leucine zipper amino acid sequence.

30

42. The polypeptide of claim 41, wherein the leucine zipper sequence comprises amino acids 231-275 of the heavy chain polypeptide amino acid sequence of Figs. 37A-37B.

43. The polypeptide of claim 35 that is an antibody fragment selected from the group consisting of Fab, Fab', Fab'-SH, Fv, scFv and F(ab')₂.

35

44. The polypeptide of claim 38 that is a F(ab')₂ antibody fragment, wherein the antibody fragment comprises a first heavy chain amino acid sequence and a second heavy chain amino acid sequence each comprising amino acids 1-238 of the heavy chain polypeptide amino acid sequence of Figs. 37A-37B, and wherein each of the Cys residues at positions 231 and 234 in the first heavy chain amino acid sequence is in a disulfide linkage with the identical Cys residue in the second heavy chain amino acid sequence.

45. The polypeptide of claim 38 that is a Fab' or Fab'-SH antibody fragment, wherein the antibody fragment comprises a heavy chain amino acid sequence comprising amino acids 1-233 of the heavy chain polypeptide amino acid sequence of Fig. 53.

46. The polypeptide of claim 35 that is an antibody.

47. A nucleic acid molecule that comprises a nucleic acid sequence encoding the polypeptide of claim 35.

48. An expression vector comprising the nucleic acid molecule of claim 47 operably linked to control sequences recognized by a host cell transfected with the vector.

49. A host cell comprising the vector of claim 48.

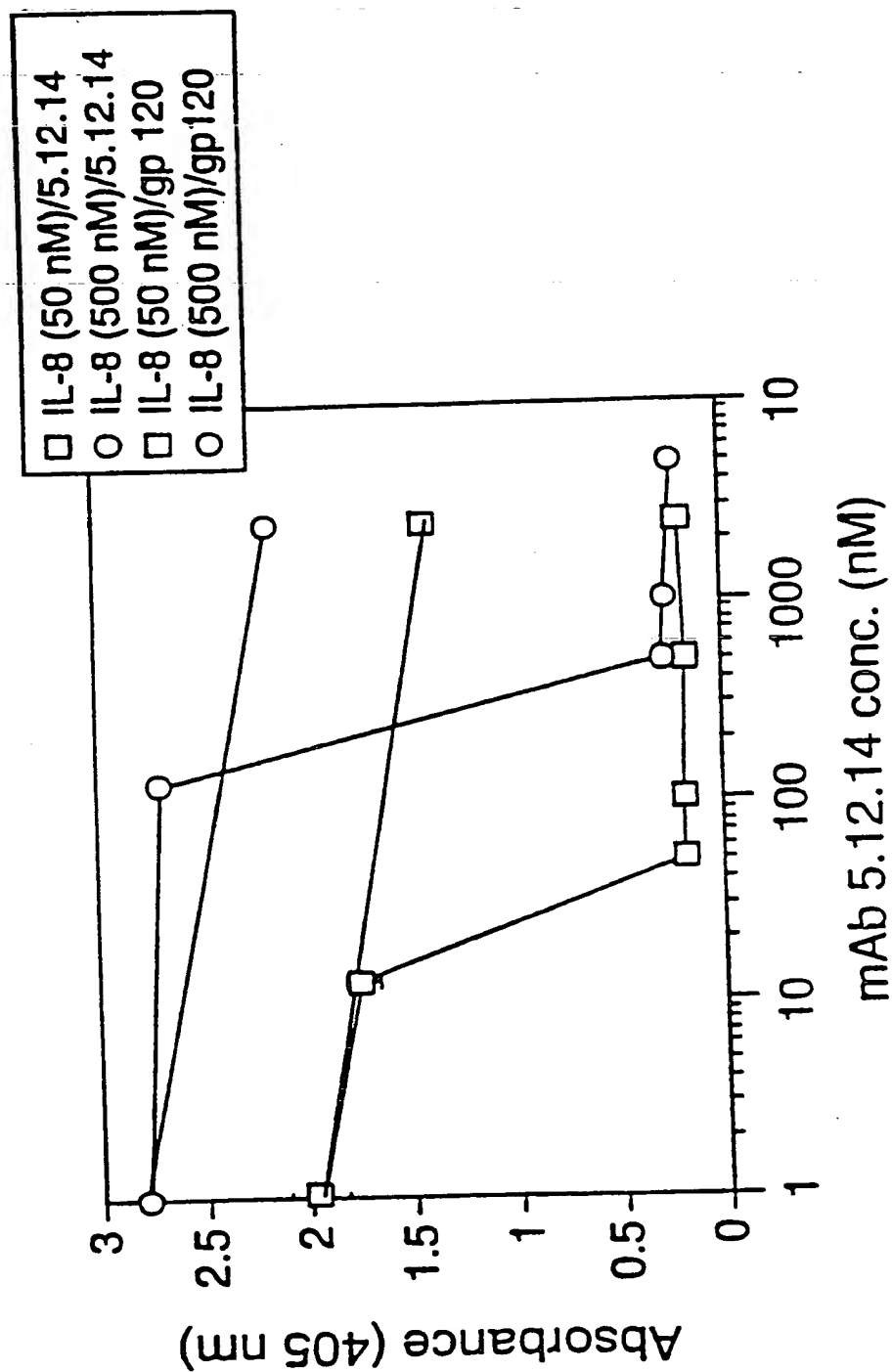
50. A method of producing a polypeptide, comprising culturing the host cell of claim 49 under conditions wherein the nucleic acid sequence is expressed, thereby producing the polypeptide, and recovering the polypeptide from the host cell.

51. A composition comprising the polypeptide of claim 35 and a carrier.

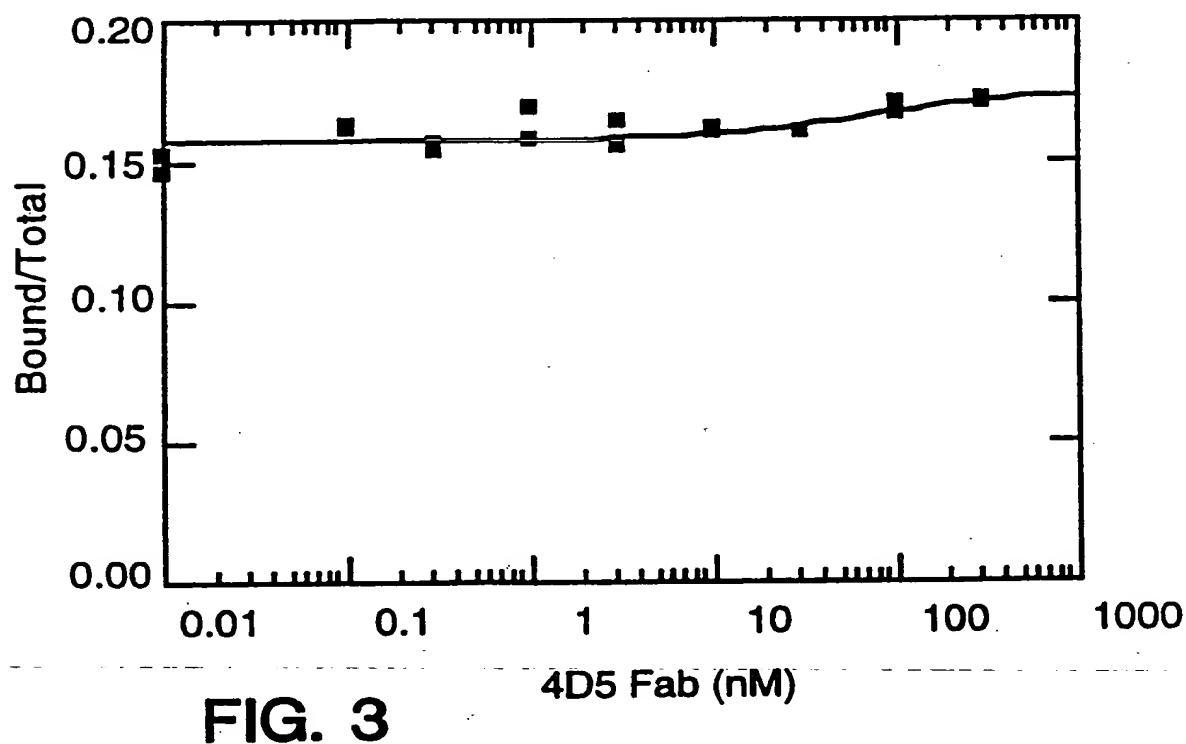
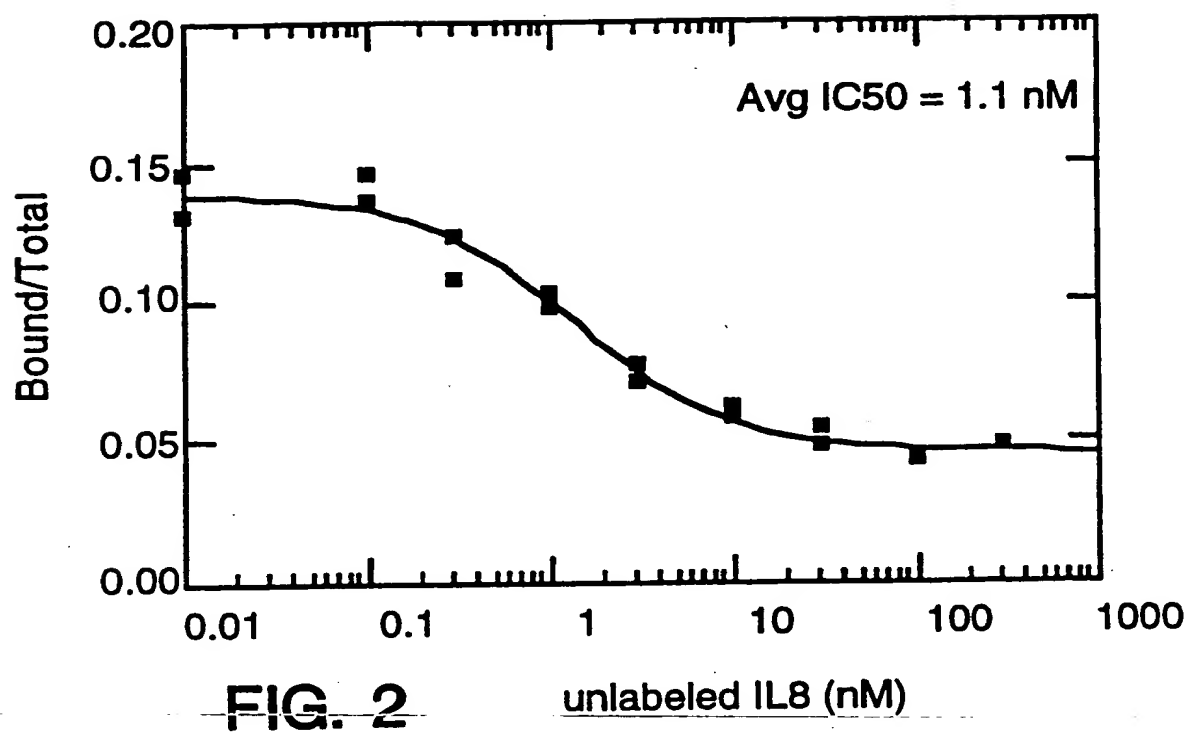
52. The composition of claim 51 that is sterile.

1 / 136

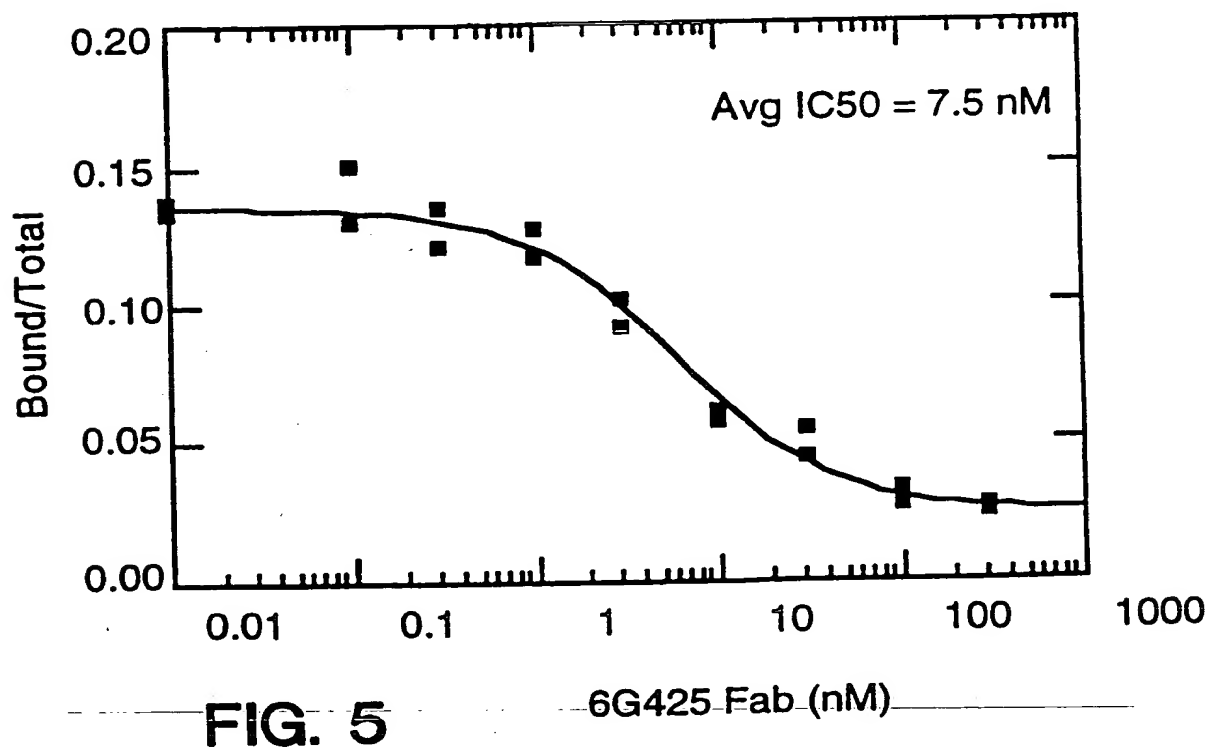
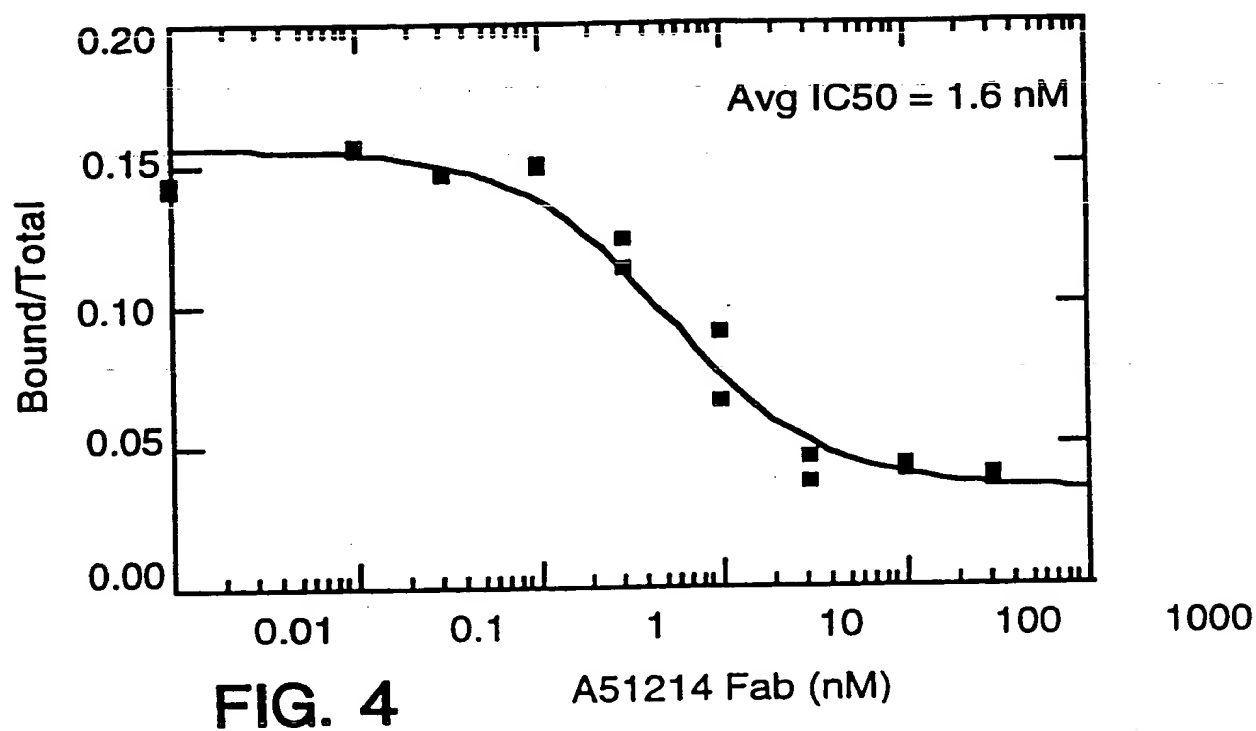
FIG. 1



2 / 136



3 / 136



4 / 136

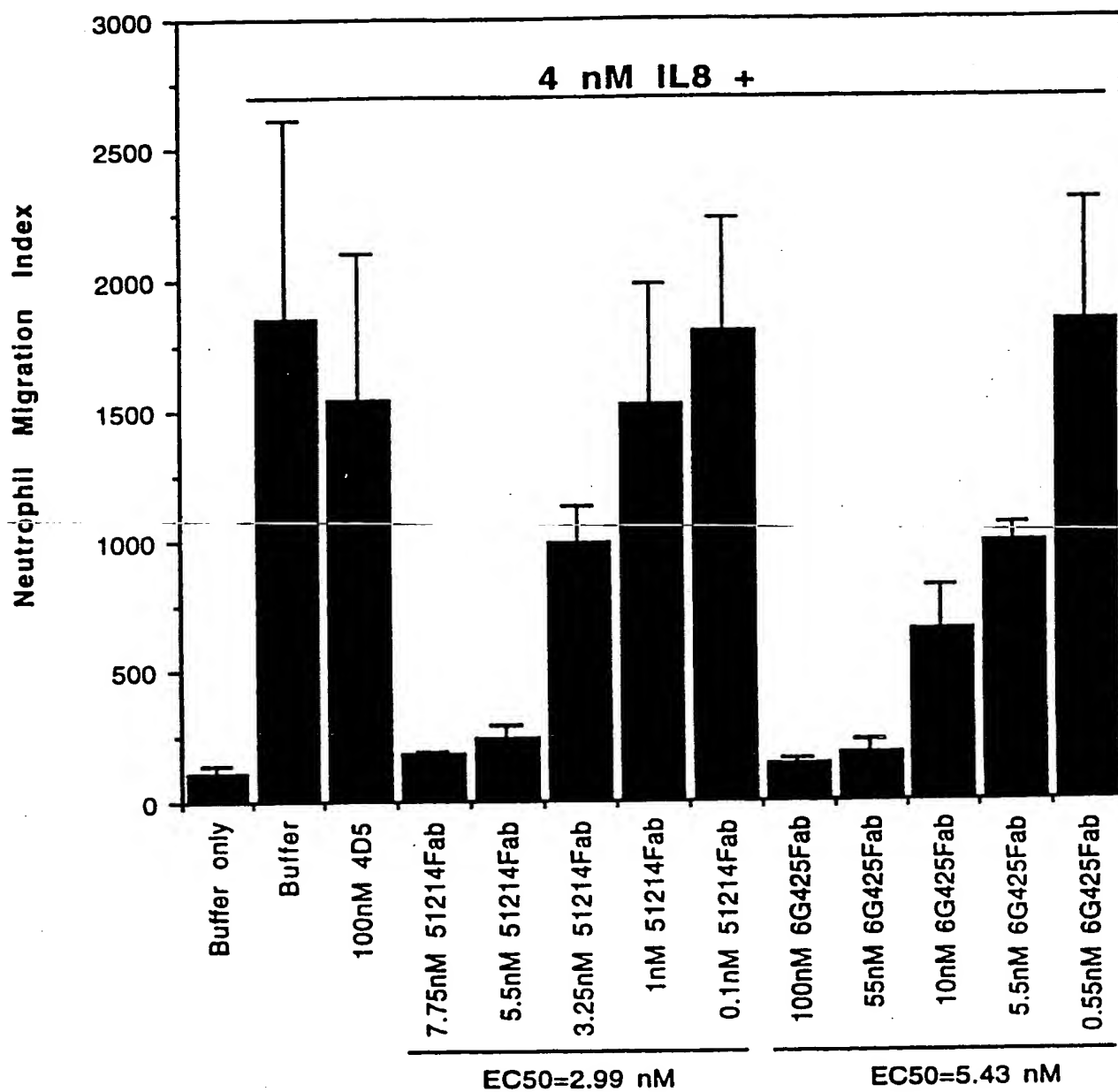


FIG. 6

5 / 136

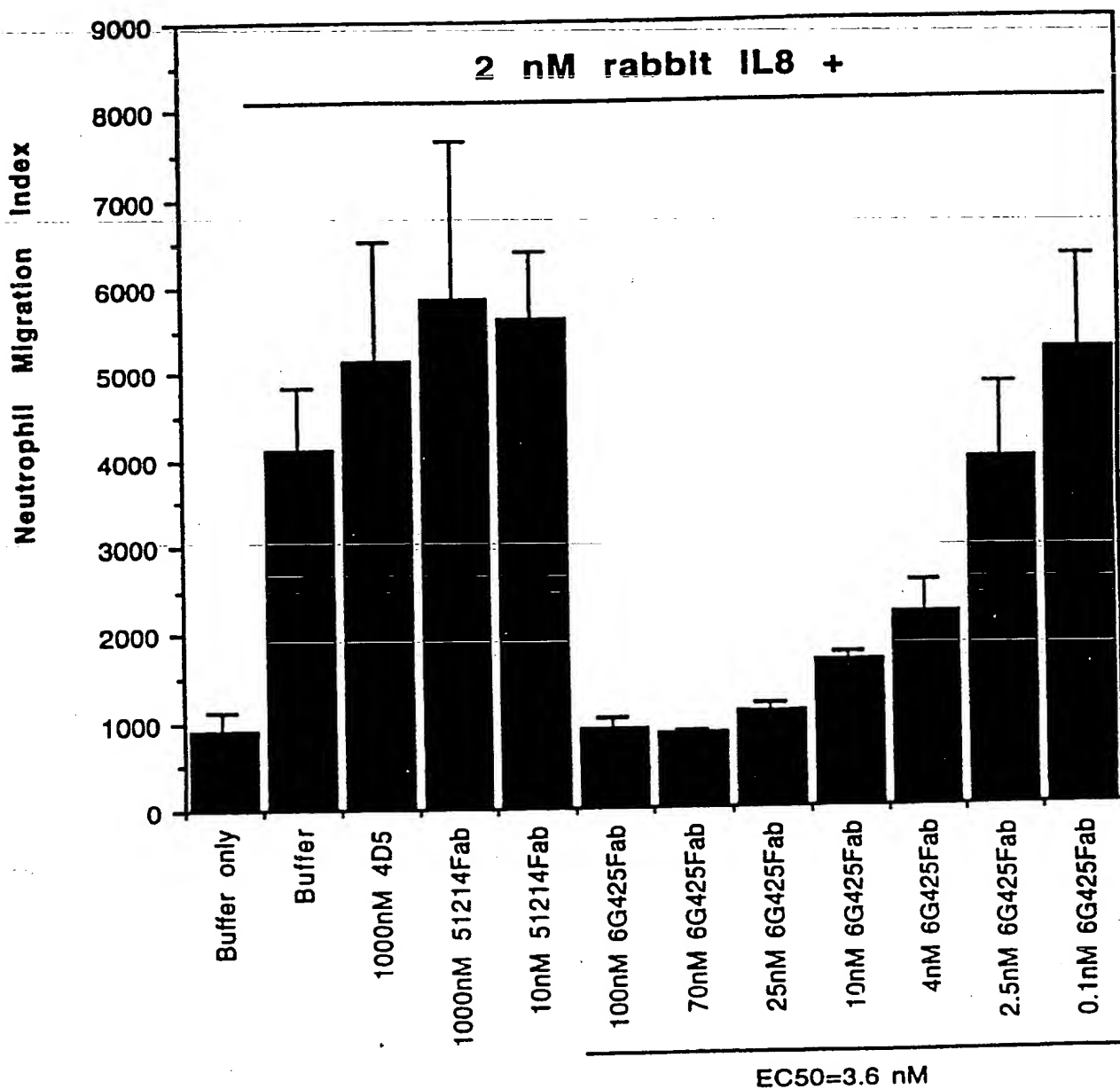
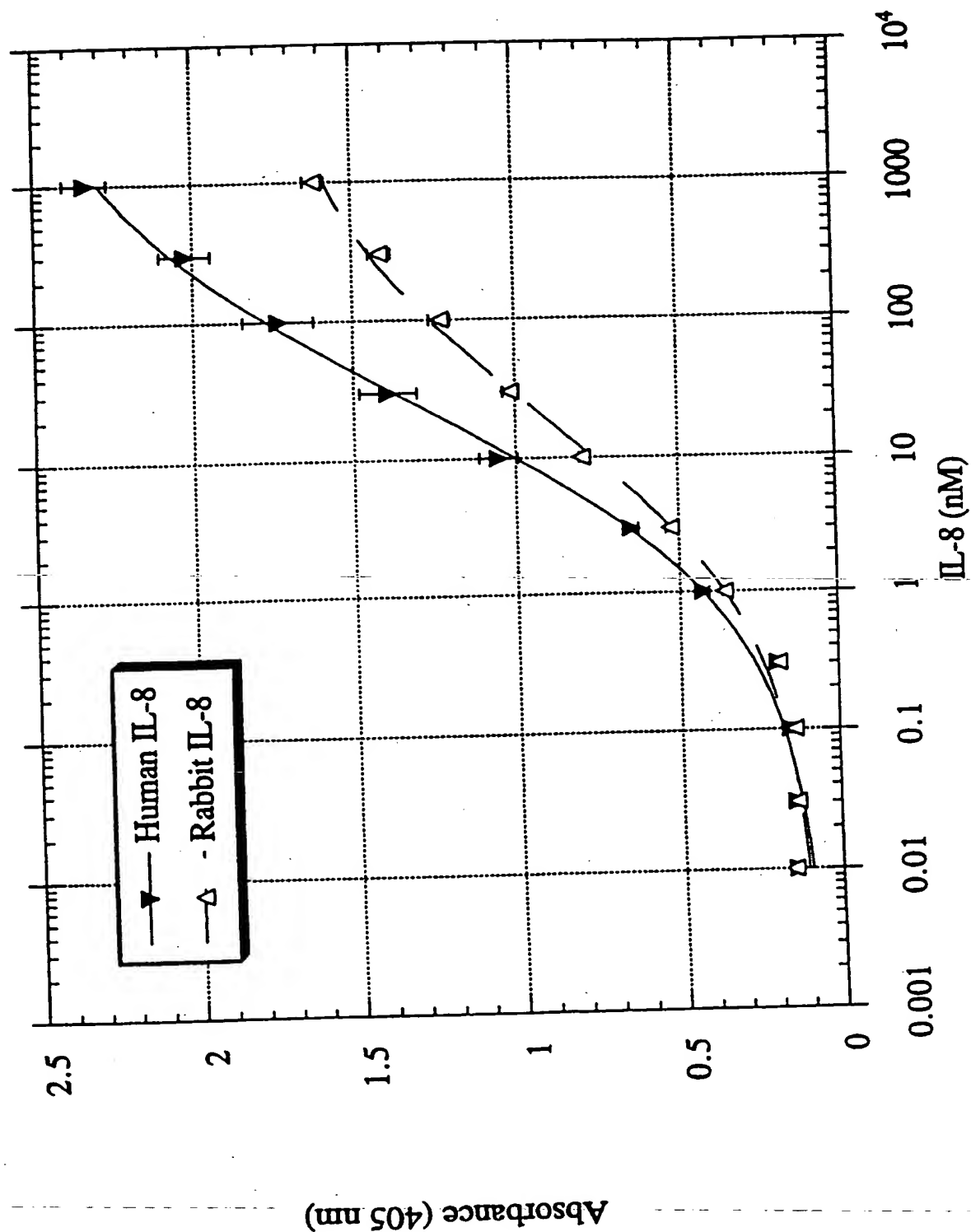


FIG. 7

6 / 136

FIG. 8



7 / 136

FIG. 9

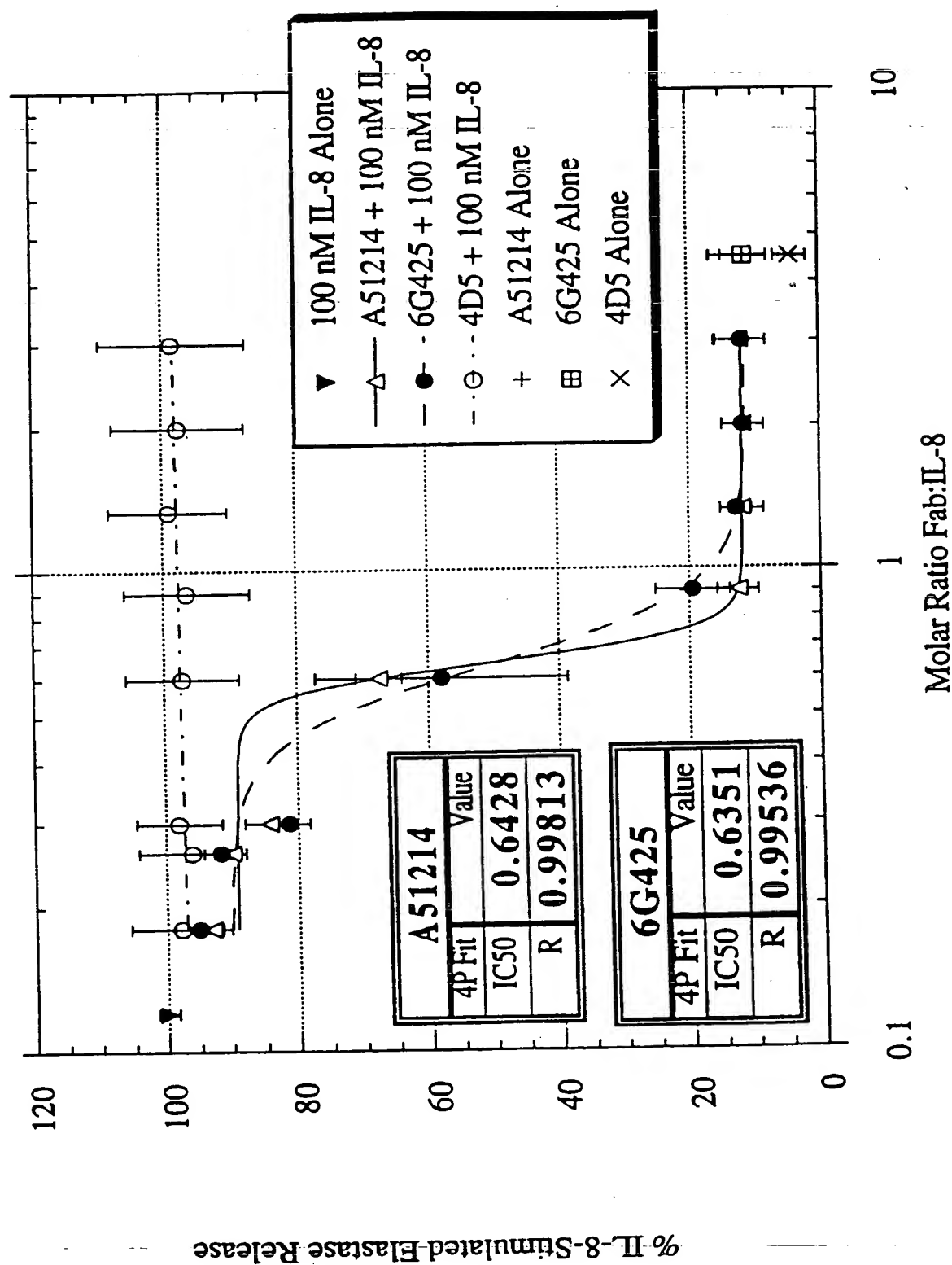
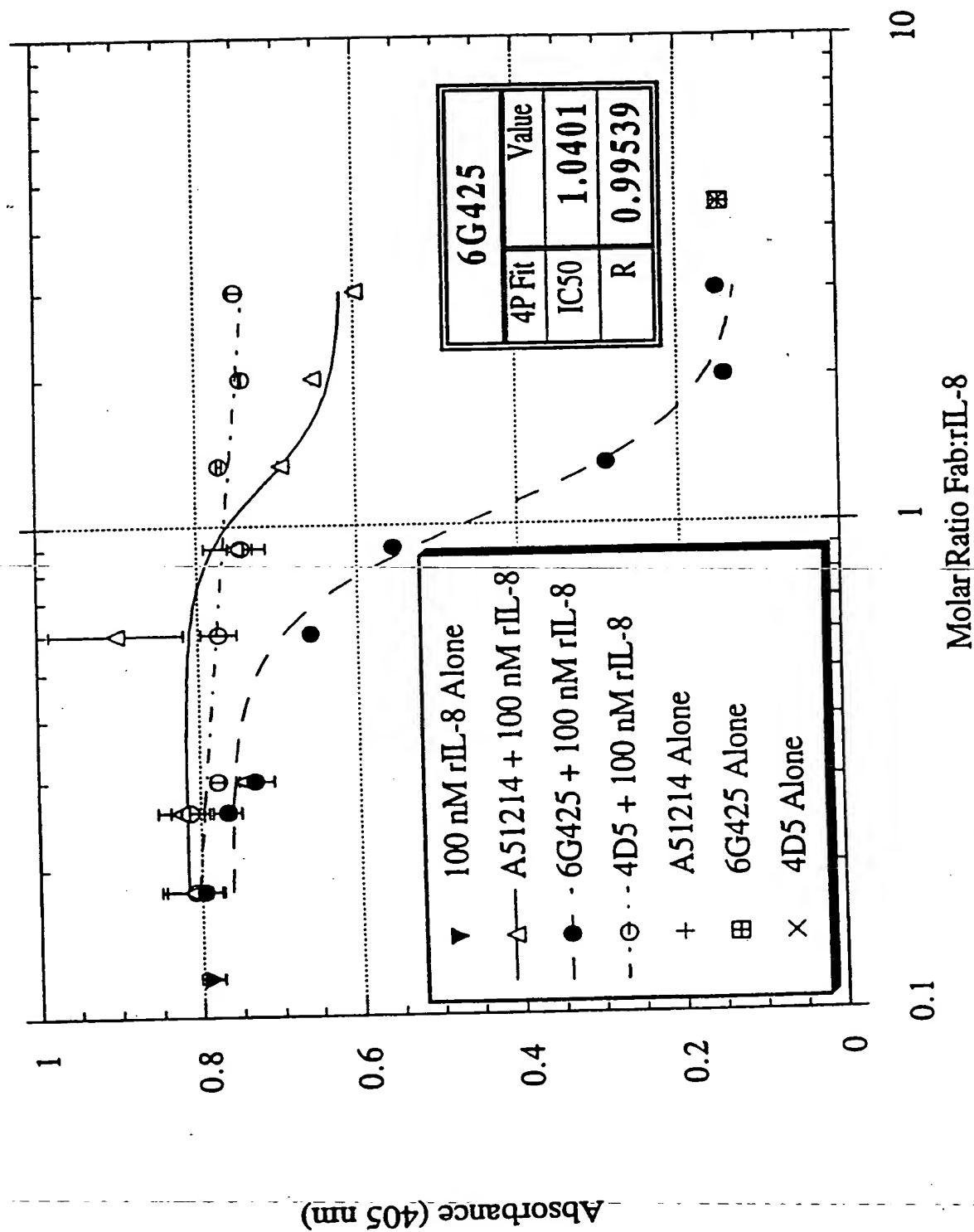


FIG. 10



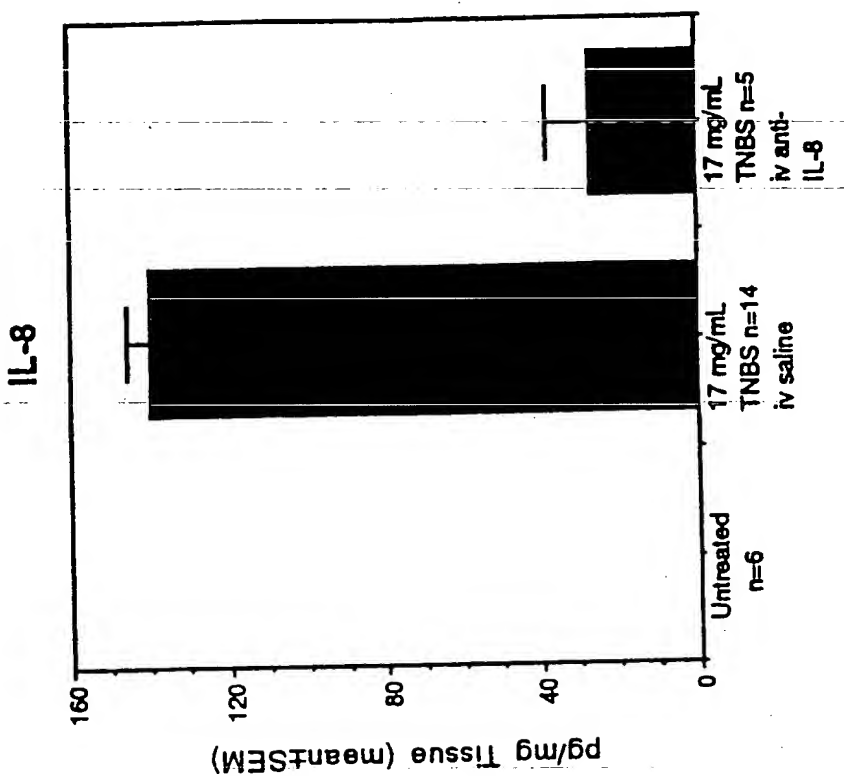


FIG. 11B

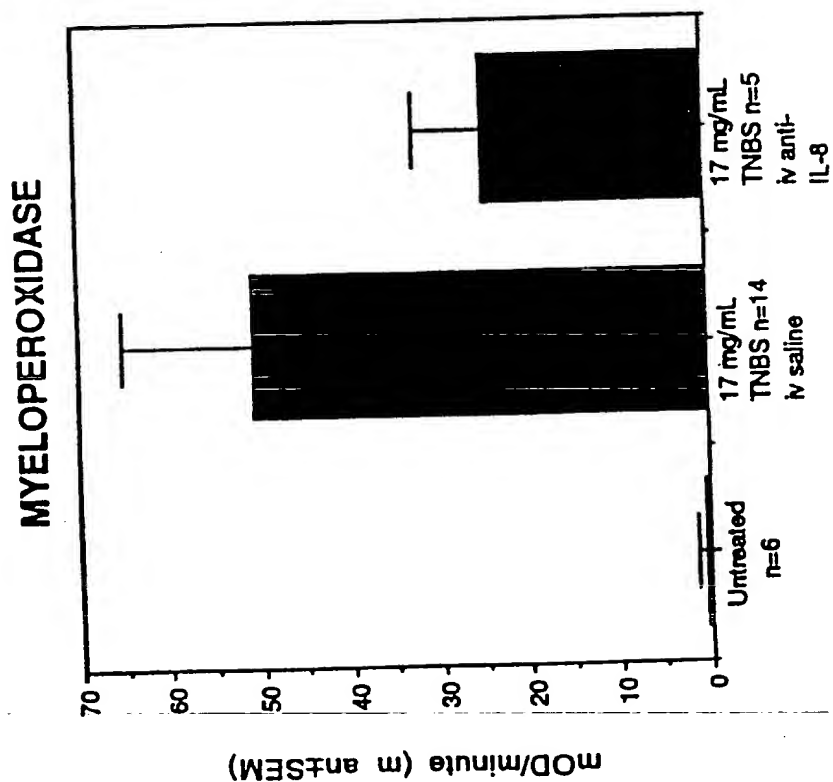


FIG. 11A

10 / 136

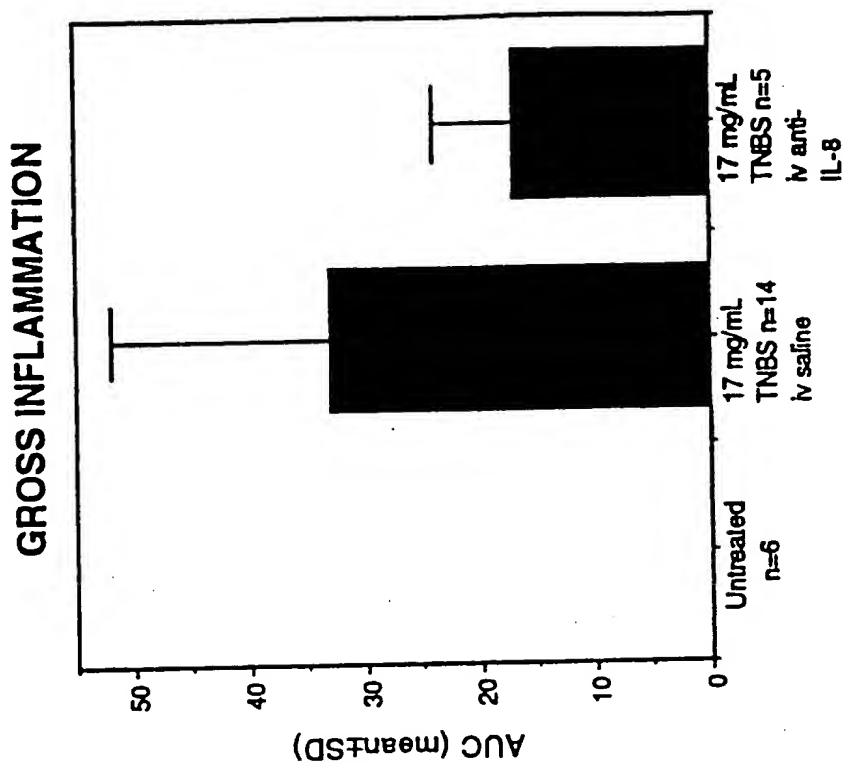


FIG. 11D

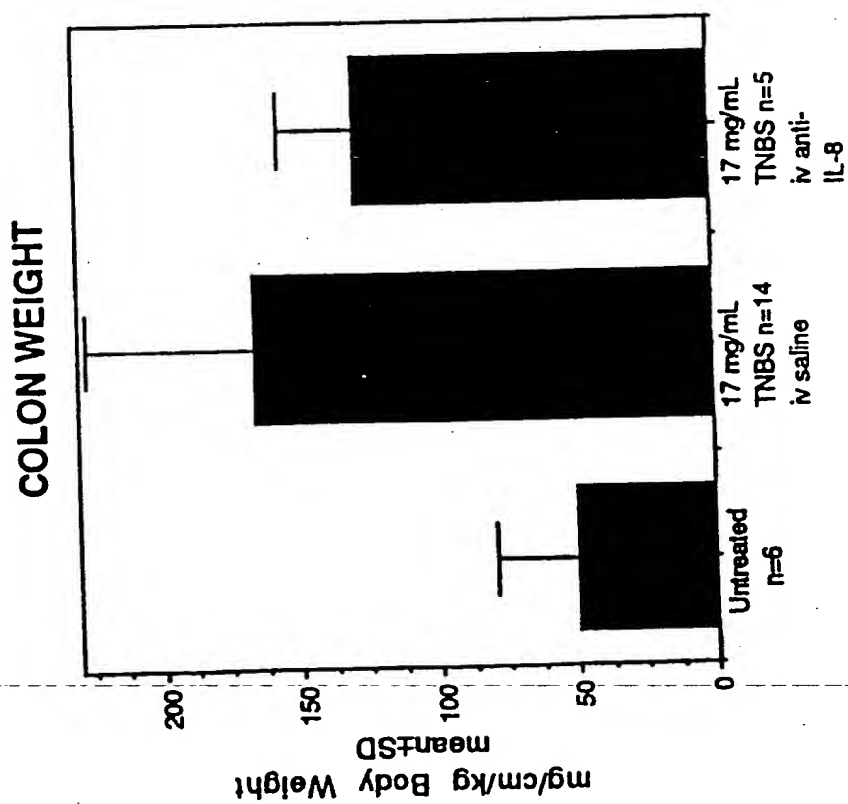


FIG. 11C

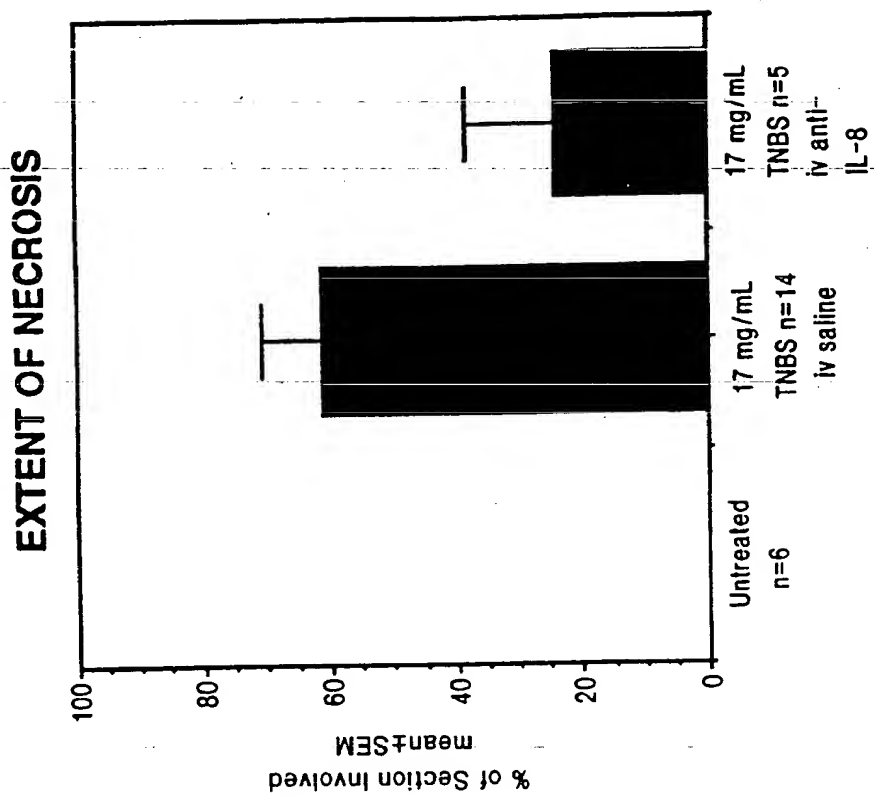


FIG. 11F

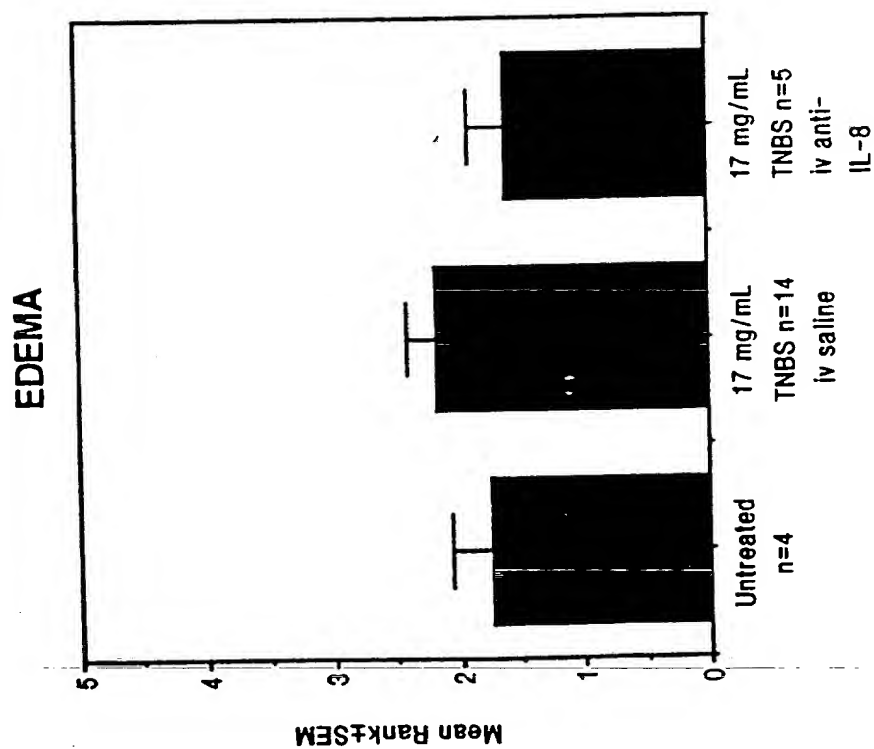


FIG. 11E

12 / 136

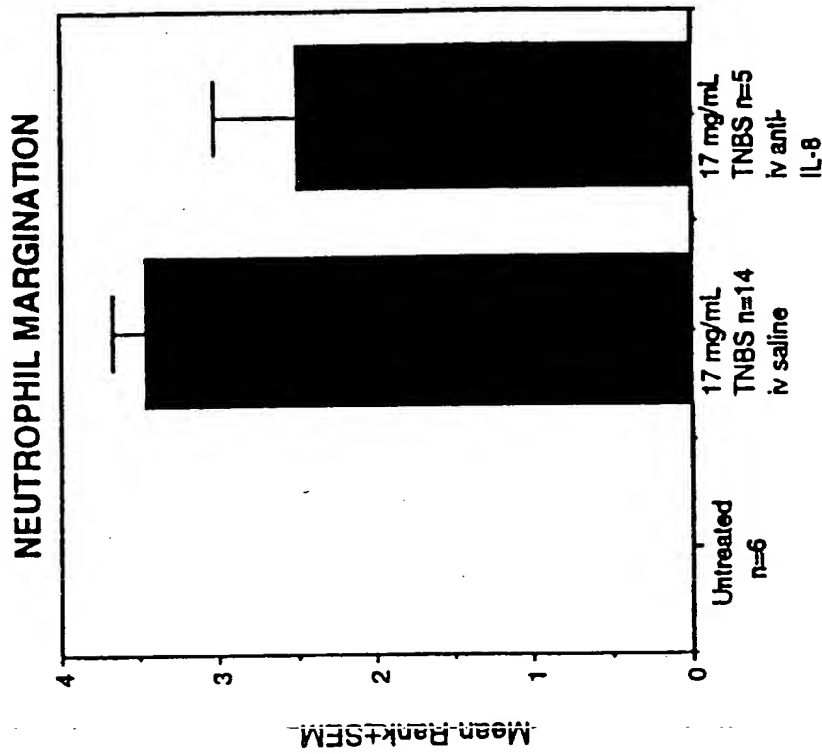


FIG. 11H

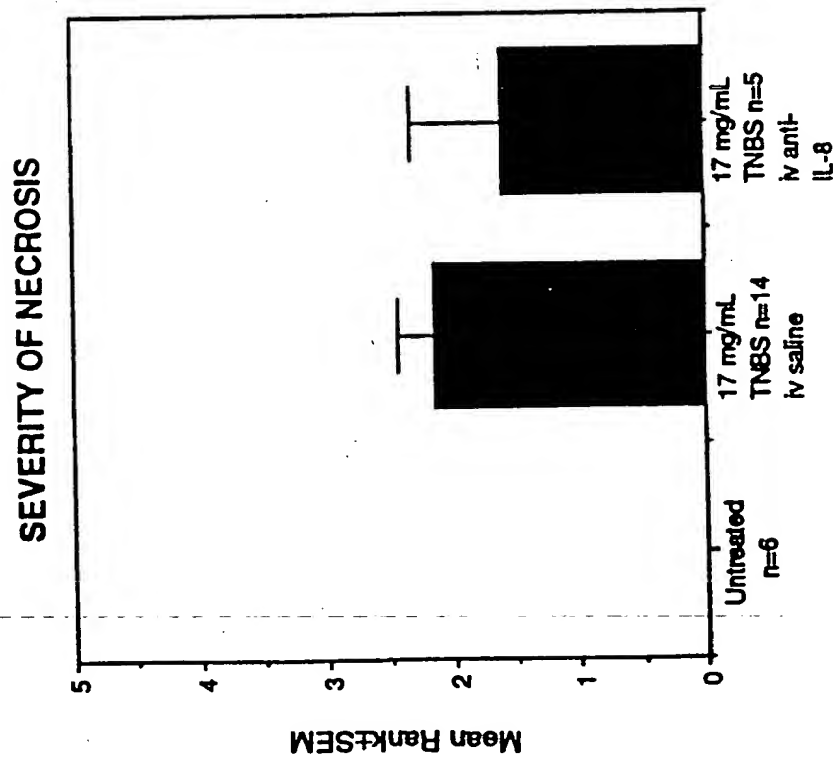


FIG. 11G

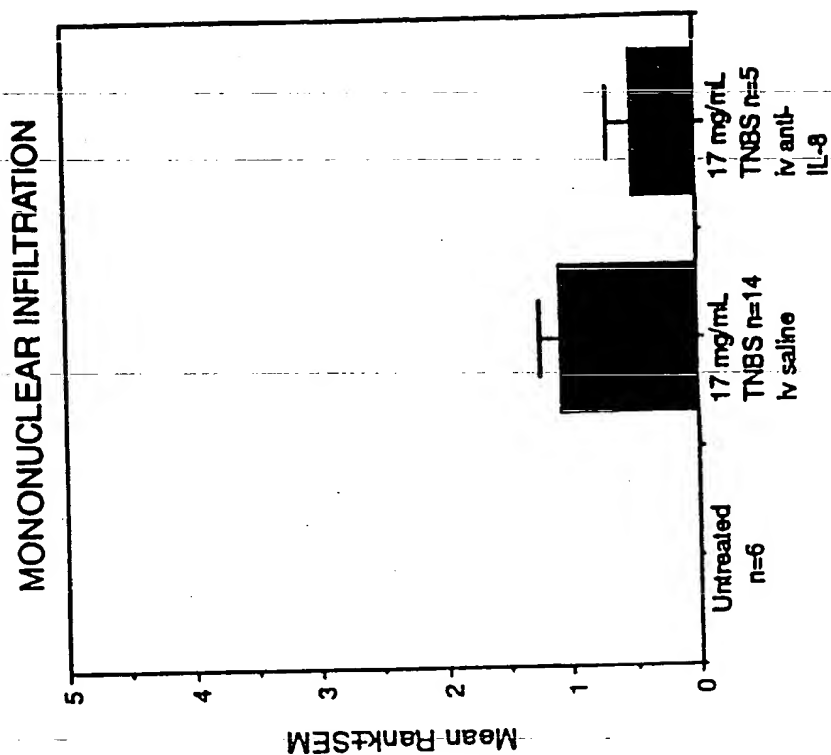


FIG. 11J

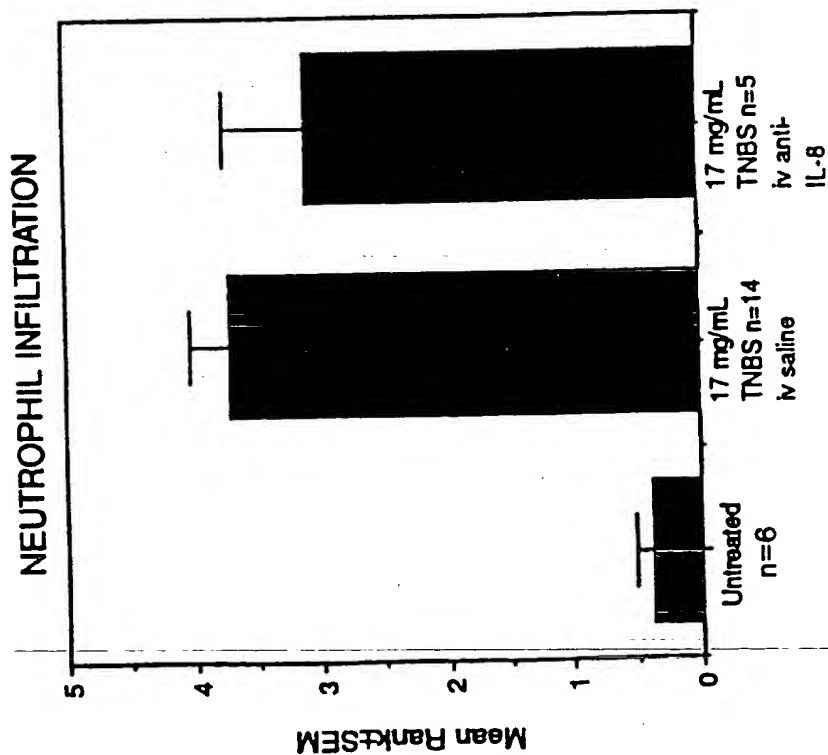
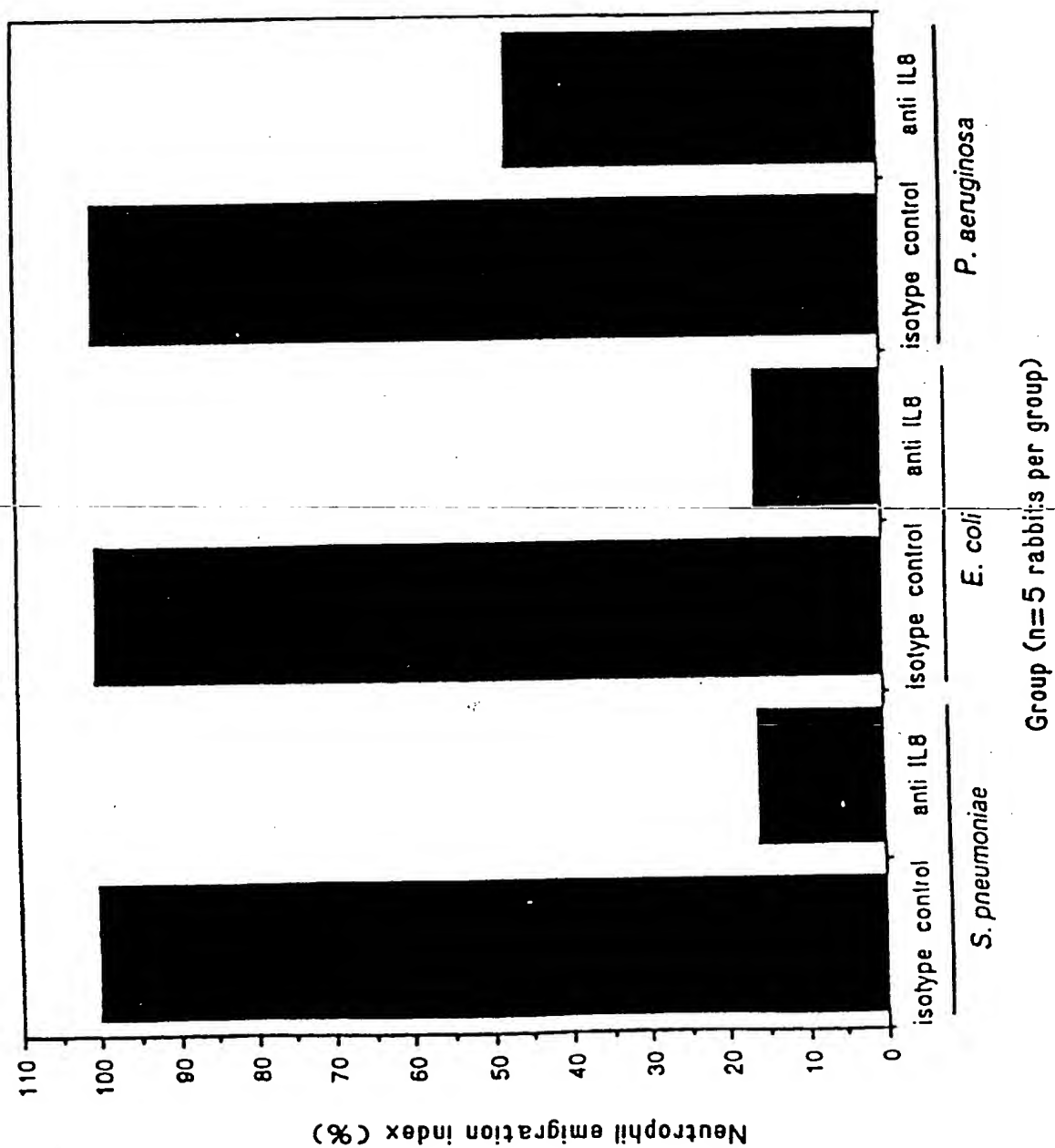


FIG. 11I

14 / 136

FIG. 12



15 / 136

Light Chain Primers:

FIG. 13

MKLC-1, 22mer

5' CAGTCCA ACTGTTCAGGACGCC 3'

MKLC-2, 22mer

5' GTGCTGCTCATGCTGTAGGTGC 3'

MKLC-3, 23mer

5' GAAGTTGATGTCTTGTGAGTGGC 3'

Heavy Chain Primers:

IGG2AC-1, 24mer

5' GCATCCTAGAGTCACCGAGGAGCC 3'

IGG2AC-2, 22mer

5' CACTGGCTCAGGGAAATAACCC 3'

IGG2AC-3, 22mer

5' GGAGAGCTGGGAAGGTGTGCAC 3'

16 / 136

FIG. 14

Light chain forward primer

~~SL001A-2 35 mer~~

5' ACAAACGCGTACGCT GACATCGTCATGACCCAGTC 3'
 T T T
 A

Light chain reverse primer

SL001B 37 mer

5' GCTCTTCGAATG GTGGGAAGATGGATACAGTTGGTGC 3'

17 / 136

Heavy chain forward primer

FIG. 15

SL002B 39 mer

5' CGATGGGCCCCG ATAGACCGATGGGGCTGTTGTTTTGGC 3'

T

C

G

A

Heavy chain reverse primer

SL002B 39-MER

5' CGATGGGCCCCG ATAGACCGATGGGGCTGTTGTTTTGGC 3'

T

A

G

18 / 136

1 GACATTGTCA TGACACAGTC TCAAAAATTC ATGTCCACAT CAGTAGGAGA CAGGGTCAGC
 CTGTAACAGT ACTGTGTCAG AGTTTTTAAG TACAGGTGTA GTCATCCTCT GTCCCAGTCG
 1 D I V M T Q S Q K F M S T S V G D R V S

61 GTCACCTGCA AGGCCAGTCA GAATGTGGGT ACTAATGTAG CCTGGTATCA ACAGAAACCA
 CAGTGGACGT TCCGGTCACT CTTACACCCA TGATTACATC GGACCATAGT TGTCCTTTGGT
 21 V T C K A S Q N V G T N V A W Y Q Q K P

CDR #1

121 GGGCAATCTC CTAAAGCACT GATTACTCG TCATCCTACC GGTACAGTGG AGTCCCTGAT
 CCCGTTAGAG GATTTCGTGA CTAATGAGC AGTAGGATGG CCATGTCACC TCAGGGACTA
 41 G Q S P K A L I Y S S Y R Y S G V P D

CDR #2

181 CGCTTCACAG GCAGTGGATC TGGACAGAT TTCACTCTCA CCATCAGCCA TGTGAGTCT
 GCGAAGTGTC CGTCACCTAG ACCCTGTCTA AAGTGAGAGT GGTAGTCGGT ACACGTCAGA
 61 R F T G S G S G T D F T L T I S H V Q S

241 GAAGACTTGG CAGACTATT CTGTCAGCAA TATAACATCT ATCCTCTCAC GTTCGGTCCT
 CTTCTGAACC GTCTGATAAA GACAGTCGTT ATATTGTAGA TAGGAGAGTG CAAAGCCAGGA
 81 E D L A D Y F C Q Q Y N I Y P L T F G P

CDR #3

301 GGGACCAAGC TGGAGTTGAA ACGGGCTGAT GCATGACCAC CAACTGTATC CATCTTCCCA
 CCCTGGTTCG ACCTCAACTT TGCCCGACTA CGACGTGGTG GTTGACATAG GTAGAAGGCT
 101 G T K L E L K R A D A A P P T V S I F P

BstBI

361 CCATTTCGAA
 GGTAAGCTT

121 P F E

FIG. 16

19 / 136

1 TTCTATTGCT ACAAACGCGT ACGCTGAGGT GCAGCTGGTG GAGTCTGGGG GAGGCTTAGT
 AAGATAACGA TGTTTGCGCA TGC GACTCCA CGTCGACCAC CTCAGACCCC CTCCGAATCA
 1 E V Q L V E S G G G L V

61 GCCGCCTGGA GGGTCCCTGA AACTCTCCTG TGCAGCCTCT GGATTTCATAT TCAGTAGTTA
 CGGCGGACCT CCCAGGGACT TTGAGAGGAC ACGTCGGAGA CCTAAGTATA AGTCATCAAT
 13 P P G G S L K L S C A A S G F I F S S Y
 * *

CDR #1

121 TGGCATGTCT TGGGTTCGCC AGACTCCAGG CAAGAGCCTG GAGTTGGTCG CAACCATTA
 ACCGTACAGA ACCCAAGCGG TCTGAGGTCC GTTCTCGGAC CTCAACCAGC GTTGGTAATT
 33 G M S W V R Q T P G K S L E L V A T I N
 * * *

181 TAATAATGGT GATAGCACCT ATTATCCAGA CAGTGTGAAG GGCCGATTCA CCATCTCCCG
 ATTATTACCA CTATCGTGGA TAATAGGTCT GTCACACTTC CCGGCTAAGT GGTAGAGGGC
 53 N N G D S T Y Y P D S V K G R F T I S R
 * * * * * * * * *

CDR #2

241 AGACAATGCC AAGAACACCC TGTACCTGCA AATGAGCAGT CTGAAGTCTG AGGACACAGC
 TCTGTTACGG TTCTTGTTGGG ACATGGACGT TTACTCGTCA GACTTCAGAC TCCTGTGTCG
 73 D N A K N T L Y L Q M S S L K S E D T A

301 CATGTTTTAC TGTGCAAGAG CCCTCATTAG TTCGGCTACT TGGTTTGTTT ACTGGGGCCA
 GTACAAAATG ACACGTTCTC GGGAGTAATC AAGCCGATGA ACCAAACCAA TGACCCCGGT
 93 M F Y C A R A L I S S A T W F G Y W G Q
 * * * * * * * * *

CDR #3

361 AGGGACTCTG GTCAGTGTCT CTGCAGCCAA AACAACAGCC CCATCTGTCT
 TCCCTGAGAC CAGTGACAGA GACGTCGGTT TTGTTGTCGG GGTAGACAGA
 113 G T L V T V S A A K T T A P S V Y

411 **ApaI**
 ATCCGGG
 TAGGCCC
 130 P

FIG. 17

20 / 136

FIG. 18

VL.front 31-MER

5' ACAAACGCGTACGCTGATATCGTCATGACAG 3'

VL.rear 31-MER

5' GCAGCATCAGCTCTTCGAAGCTCCAGCTTGG 3'

VH.front.SPE 21-MER

5' CCACTAGTACGCAAGTTCACG 3'

VH.rear 33-MER

5' GATGGGCCCTTGGTGGAGGCTGCAGAGACAGTG 3'

21 / 136

1 ATGAAGAAGA ATATCGCATT TCTTCTTGCA TCTATGTTCTG TTTTCTCTAT TGCTACAAAC
TACTTCTTCT TATAGCGTAA AGAAGAACGT AGATACAAGC AAAAAAGATA ACGATGTTTG
-23 M K K N I A F L L A S M F V F S I A T N

61 GCGTACGCTG ATATCGTCAT GACACAGTCT CAAAAATTCA TGTCCACATC AGTAGGAGAC
CGCATGCGAC TATAGCAGTA CTGTGTCAGA GTTTTAAAGT ACAGGTGTAG TCATCCTCTG
-3 A Y A D I V M T Q S Q K F M S T S V G D

121 AGGGTCAGCG TCACCTGCAA GGCCAGTCAG AATGTGGGTA CTAATGTAGC CTGGTATCAA
TCCCAGTCGC AGTGGACGTT CCGGTCAGTC TTACACCCAT GATTACATCG GACCATAGTT
18 R V S V T C K A S O N V G T N V A W Y Q
* * * * *

CDR #1

181 CAGAAACCAG GGCAATCTCC TAAAGCACTG ATTTACTCGT CATCCTACCG GTACAGTGGA
GTCTTTGGTC CCGTTAGAGG ATTTCTGTGAC TAAATGAGCA GTAGGATGGC CATGTCACTT
38 Q K P G Q S P K A L I Y S S S Y R Y S G
* * * * *

CDR #2

241 GTCCCTGATC GCTTCACAGG CAGTGGATCT GGGACAGATT TCACTCTCAC CATCAGCCAT
CAGGGACTAG CGAAGTGTCC GTCACCTAGA CCCTGTCATA AGTGAGAGTG GTAGTCGGTA
58 V P D R F T G S G S G T D F T L T I S H

301 GTGCAGTCTG AAGACTTGGC AGACTATTTT TGTCAGCAAT ATAACATCTA TCCTCTCAGG
CACGTCAGAC TTCTGAACCG TCTGATAAAG ACAGTCGTTA TATTGTAGAT AGGAGAGTGC
78 V Q S E D L A D Y F C Q Q Y N I Y P L T
* * * * *

CDR #3

BstBI

361 TTCGGTCTTG GGACCAAGCT GGAGCTTCGA AGAGCTGTGG CTGCACCATC TGTCTTCATC
AAGCCAGGAC CCTGGTTCGA CCTCGAAGCT TCTCGACACC GACGTGGTAG ACAGAAGTAG
98 F G P G T K L E L R R A V A A P S V F I

421 TTCCCGCCAT CTGATGAGCA GTTGAAATCT GGAAGTGTCT CTGTTGTGTG CCTGCTGAAT
AAGGGCGGTA GACTACTCGT CAACTTTAGA CCTTGACGAA GACAACACAC GGACGACTTA
118 F P P S D E Q L K S G T A S V V C L L N

481 AACTTCTATC CCAGAGAGGC CAAAGTACAG TGGAAGGTGG ATAACGCCCT CCAATCGGGT
TTGAAGATAG GGTCTCTCCG GTTTCATGTC ACCTTCCACC TATTGCGGGA GGTTAGCCCA
138 N F Y P R E A K V Q W K V D N A L Q S G

541 AACTCCCAGG AGAGTGTCAC AGAGCAGGAC AGCAAGGACA GCACCTACAG CCTCAGCAGC
TTGAGGGTCC TCTCACAGTG TCTCGTCCTG TCGTTCCTGT CGTGGATGTC GGAGTCGTCG
158 N S Q E S V T E Q D S K D S T Y S L S S

601 ACCCTGACGC TGAGCAAAGC AGACTACGAG AAACACAAAG TCTACGCCTG CGAAGTCACC
TGGGACTGCG ACTCGTTTCG TCTGATGCTC TTTGTGTTTC AGATGCGGAC GCTTCAGTGG
178 T L T L S K A D Y E K H K V Y A C E V T

661 CATCAGGGCC TGAGCTCGCC CGTCACAAAG AGCTTCAACA GGGGAGAGTG
GTAGTCCCGG ACTCGAGCGG GCAGTGTTC TCGAAGTTGT CCCCTCTCAC
198 H Q G L S S P V T K S F N R G E C

711 TTAA
AATT
216 O

FIG. 19

22 / 136

```

1  ATGAAAAAGA ATATCGCATT TCTTCTTGCA TCTATGTTCG TTTTCTCTAT TGCTACAAAC
   TACTTTTTCT TATAGCGTAA AGAAGAACGT AGATACAAGC AAAAAAGATA ACGATGTTTG
-23 M K K N I A F L L A S M F V F S I A T N

61  GCGTACGCTG AGGTGCAGCT GGTGGAGTCT GGGGGAGGCT TAGTGCCGCC TGGAGGGTCC
   CGCATGCGAC TCCACGTCGA CCACCTCAGA CCCCCTCCGA ATCACGGCGG ACCTCCCAGG
-3  A Y A E V Q L V E S G G G L V P P G G S

121 CTGAAACTCT CCTGTGCAGC CTCTGGATTCT ATATTTCAGTA GTTATGGCAT GTCTTGGGTT
   GACTTTGAGA GGACACGTCG GAGACCTAAG TATAAGTCAT CAATACCGTA CAGAACCCAA
18  L K L S C A A S G F I F S S Y G M S W V
                        * * * * *

```

CDR #1

```

181 CGCCAGACTC CAGGCAAGAG CCTGGAGTTG GTCGCAACCA TTAATAATAA TGGTGATAGC
   GCGGTCTGAG GTCCGTTCTC GGACCTCAAC CAGCGTTGGT AATTATTATT ACCACTATCG
38  R Q T P G K S L E L V A T I N N N G D S
                        * * * * *

```

```

241 ACCTATTATC CAGACAGTGT GAAGGGCCGA TTCACCATCT CCCGAGACAA TGCCAAGAAC
   TGGATAATAG GTCTGTCACA CTTCCCGGCT AAGTGGTAGA GGGCTCTGTT ACGGTTCTTG
58  T Y Y P D S V K G R F T I S R D N A K N
   * * * * *

```

CDR #2

```

301 ACCCTGTACC TGCAAATGAG CAGTCTGAAG TCTGAGGACA CAGCCATGTT TTACTGTGCA
   TGGGACATGG ACGTTTACTC GTCAGACTTC AGACTCCTGT GTCGGTACAA AATGACACGT
78  T L Y L Q M S S L K S E D T A M F Y C A

361 AGAGCCCTCA TTAGTTCGGC TACTTGGTTT GGTTACTGGG GCCAAGGGAC TCTGGTCACT
   TCTCGGGAGT AATCAAGCCG ATGAACCAAA CCAATGACCC CGGTTCCCTG AGACCAGTGA
98  R A L I S S A T W F G Y W G Q G T L V T
   * * * * *

```

CDR #3

ApaI

```

421 GTCTCTGCAG CCTCCACCAA GGGCCCATCG GTCTTCCCCC TGGCACCCCTC CTCCAAGAGC
   CAGAGACGTC GGAGGTGGTT CCCGGGTAGC CAGAAGGGGG ACCGTGGGAG GAGGTTCTCG
118 V S A A S T K G P S V F P L A P S S K S

481 ACCTCTGGGG GCACAGCGGC CCTGGGCTGC CTGGTCAAGG ACTACTTCCC CGAACC GGGTG
   TGGAGACCCC CGTGTGCGCC GGACCCGACG GACCAGTTCC TGATGAAGGG GCTTGGCCAC
138 T S G G T A A L G C L V K D Y F P E P V

541 ACGGTGTCGT GGAAGTCAGG CGCCCTGACC AGCGGCGTGC ACACCTTCCC GGCTGTCCTA
   TGCCACAGCA CCTTGAGTCC GCGGGACTGG TCGCCGCACG TGTGGAAGGG CCGACAGGAT
158 T V S W N S G A L T S G V H T F P A V L

601 CAGTCCTCAG GACTCTACTC CCTCAGCAGC GTGGTGACCG TGCCCTCCAG CAGCTTGGGC
   GTCAGGAGTC CTGAGATGAG GGAGTCGTCG CACCACTGGC ACGGGAGGTC GTCGAACCCG
178 Q S S G L Y S L S S V V T V P S S S L G

```

FIG. 20A

23 / 136

661 ACCCAGACCT ACATCTGCAA CGTGAATCAC AAGCCCAGCA ACACCAAGGT GGACAAGAAA
TGGGTCTGGA TGTAGACGTT GCACTTAGTG TTCGGGTCGT TGTGGTTCCA CCTGTTCTTT
198 T Q T Y I C N V N H K P S N T K V D K K

721 GTTGAGCCCA AATCTTGTGA CAAAACTCAC ACATGA
CAACTCGGGT TTAGAACACT GTTTTGAGTG TGTACT
218 V E P K S C D K T H T O

FIG. 20B

24 / 136

Light Chain Primers:

MKLC-1, 22mer

5' CAGTCCAAGTGTTCAGGACGCC 3'

MKLC-2, 22mer

5' GTGCTGCTCATGCTGTAGGTGC 3'

MKLC-3, 23mer

5' GAAGTTGATGTCTTGTGAGTGGC 3'

Heavy Chain Primers:

IGG2AC-1, 24mer

5' GCATCCTAGAGTCACCGAGGAGCC 3'

IGG2AC-2, 22mer

5' CACTGGCTCAGGGAAATAACCC 3'

IGG2AC-3, 22mer

5' GGAGAGCTGGGAAGGTGTGCAC 3'

FIG. 21

25 / 136

Light chain forward primer

6G4.light.Nsi 36-MER

5' CCAATGCATACGCT GAC ATC GTG ATG ACC CAG ACC CC 3'
 T T T T
 A A

Light chain reverse primer

6G4.light.Mun 35-MER

5' AGA TGT CAA TTG CTC ACT GGA TGG TGG GAA GAT GG 3'

FIG. 22

26 / 136

Heavy chain forward primer

6G4.heavy.Mlu 32-MER

5' CAAACGCGTACGCT GAG ATC CAG CTG CAG CAG 3'
 T C

Heavy chain reverse primer

SL002B 39-MER

5' CGATGGGCCCCG ATAGACCGATGGGGCTGTTGTTTGGC 3'
 T
 A
 G

FIG. 23

27/136

70 G ATATCGTGAT GACACAGACA CCACTCTCCC TGCCTGTCAG TCTTGGAGAT
 C TATAGCACTA CTGTGTCTGT GGTGAGAGGG ACGGACAGTC AGAACCTCTA
 1 D I V M T Q T P L S L P V S L G D

121 CAGGCCTCCA TCTCTTGCAG ATCTAGTCAG AGCCTTGATC ACGGTATTGG AAACACCTAT
 GTCCGGAGGT AGAGAACGTC TAGATCAGTC TCGGAACATG TGCCATAACC TTTGTGGATA
 18 Q A S I S C R S S O S L V H G I G N T Y
 * * * * *

CDR #1

181 TTACATTGGT ACCTGCAGAA GCCAGGCCAG TCTCCAAAGC TCCTGATCTA CAAAGTTTCC
 AATGTAACCA TGGACGTCTT CCGTCCGGTC AGAGGTTTCG AGGACTAGAT GTTTCAAAGG
 38 L H W Y L Q K P G Q S P K L L I Y K V S
 * * * * *

CDR #2

241 AACCGATTTT CTGGGGTCCC AGACAGGTTC AGTGGCAGTG GATCAGGGAC AGATTTTACA
 TTGGCTAAAA GACCCAGGG TCTGTCCAAG TCACCGTCAC CTAGTCCCTG TCTAAAGTGT
 58 N R F S G V P D R F S G S G S G T D F T
 * * * * *

301 CTCAGGATCA GCAGAGTGGA GGCTGAGGAT CTGGGACTTT ATTTCTGCTC TCAAAGTACA
 GAGTCCTAGT CGTCTCACCT CCGACTCCTA GACCCTGAAA TAAAGACGAG AGTTTCATGT
 78 L R I S R V E A E D L G L Y F C S Q S T
 * * * * *

CDR #3

361 CATGTTCCGC TCACGTTCGG TGCTGGGACC AAGCTGGAGC TGAAACGGGC TGATGCTGCA
 GTACAAGGCG AGTGCAAGCC ACGACCCTGG TTCGACCTCG ACTTTGCCCC ACTACGACGT
 98 H V P L T F G A G T K L E L K R A D A A
 * * * * *

MunI

421 CCAACTGTAT CCATCTTCCC ACCATCCAGT GAGCAATTGA
 GGTGACATA GGTAGAAGGG TGGTAGGTCA CTCGTAACT
 118 P T V S I F P P S S E Q L K

FIG. 24

28 / 136

70 G AGATTCAGCT GCAGCAGTCT GGACCTGAGC TGATGAAGCC TGGGGCTTCA
 C TCTAAGTCGA CGTCGTCAGA CCTGGACTCG ACTACTTCGG ACCCCGAAGT
 1 E I Q L Q Q S G P E L M K P G A S

121 GTGAAGATAT CCTGCAAGGC TTCTGGTTAT TCATTGAGTA GCCACTACAT GCACTGGGTG
 CACTTCTATA GGACGTTCCG AAGACCAATA AGTAAGTCAT CGGTGATGTA CGTGACCCAC
 18 V K I S C K A S G Y S F S S H Y M H W V
 * * * * *

CDR #1

181 AAGCAGAGCC ATGGAAAGAG CCTTGAGTGG ATTGGCTACA TTGATCCTTC CAATGGTGAA
 TTCGTCTCGG TACCTTTCTC GGAAGTACAC TAACCGATGT AACTAGGAAG GTTACCACTT
 38 K Q S H G K S L E W I G Y I D P S N G E
 * * * * *

CDR #2

241 ACTACTTACA ACCAGAAATT CAAGGGCAAG GCCACATTGA CTGTAGACAC ATCTTCCAGC
~~TGATGAATGT TGGTCTTTAA GTTCCCGTTC CGGTGTAAC~~ ~~GACATCTGTG TAGAAGGTGC~~
 58 T T Y N Q K F K G K A T L T V D T S S S
 * * * * *

301 ACAGCCAACG TGCATCTCAG CAGCCTGACA TCTGATGACT CTGCAGTCTA TTTCTGTGCA
 TGTCGGTTGC ACGTAGAGTC GTCGGACTGT AGACTACTGA GACGTCAGAT AAAGACACGT
 78 T A N V H L S S L T S D D S A V Y F C A

361 AGAGGGGACT ATAGATACAA CGGCGACTGG TTTTTCGATG TCTGGGGCGC AGGGACCACG
 TCTCCCTGTA TATCTATGTT GCCGCTGACC AAAAAGCTAC AGACCCCGCG TCCCTGGTGC
 98 R G D Y R Y N G D W F F D V W G A G T T
 * * * * *

CDR #3

BstEII ApaI
 421 GTCACCGTCT CCTCCGCCAA AACCGACAGC CCCATCGGTC TATCCGGGCC
 CAGTGGCAGA GGAGGCGGAT TTGGCTGTCT GGGTAGCCAG ATAGGCCCGG
 118 V T V S S A K T D S P I G L S G P

471 CATC
 GTAG

135 I

FIG. 25

29 / 136

5' CTTGGTGGAGGCGGAGGAGACG 3'

Mutagenesis Primer for 6G425VL

DS/VF 38MER

5' GAAACGGGCTGTTGCTGCACCAACTGTATTCATCTTCC 3'

SYN.BstEII 31 MER

5' GTCACCGTCT CCTCCGCCTC CACCAAGGGC C 3'

SYN.Apa 22 MER

5' CTTGGTGGAGGCGGAGGAGACG 3'

FIG. 26

30 / 136

1 ATGAAGAAGA ATATCGCATT TCTTCTTGCA TCTATGTTTCG TTTTTCCTAT TGCTACAAAT
TACTTCTTCT TATAGCGTAA AGAAGAACGT AGATACAAGC AAAAAAGATA ACGATGTTTA
-23 M K K N I A F L L A S M F V F S I A T N

61 GCATACGCTG ATATCGTGAT GACACAGACA CCACTCTCCC TGCCTGTCAG TCTTGAGAT
CGTATGCGAC TATAGCACTA CTGTGTCTGT GGTGAGAGGG ACGGACAGTC AGAACCTCTA
-3 A Y A D I V M T Q T P L S L P V S L G D

121 CAGGCCTCCA TCTCTTGCA GATCTAGTCAG AGCCTTGTAC ACGGTATTGG AAACACCTAT
GTCCGGAGGT AGAGAACGTC TAGATCAGTC TCGGAACATG TGCCATAACC TTTGTGGATA
18 Q A S I S C R S S O S L V H G I G N T Y
* * * * *

CDR #1

181 TTACATTGGT ACCTGCAGAA GCCAGGCCAG TCTCCAAAGC TCCTGATCTA CAAAGTTTCC
AATGTAACCA TGGACGTCTT CGGTCCGGTC AGAGGTTTCG AGGACTAGAT GTTTCAAAGG
38 L H W Y L Q K P G Q S P K L L I Y K V S
* * * * *

CDR #2

241 AACCGATTTT CTGGGGTCCC AGACAGGTC AGTGGCAGTG GATCAGGGAC AGATTTTACA
TTGGCTAAAA GACCCAGGG TCTGTCCAAG TCACCGTCAC CTAGTCCCTG TCTAAAGTGT
58 N R F S G V P D R F S G S G S G T D F T
* * * *

301 CTCAGGATCA GCAGAGTGGA GGCTGAGGAT CTGGGACTTT ATTTCTGCTC TCAAAGTACA
GAGTCCTAGT CGTCTCACCT CCGACTCCTA GACCCTGAAA TAAAGACGAG AGTTTCATGT
78 L R I S R V E A E D L G L Y F C S Q S T
* * * *

CDR #3

361 CATGTTCCGC TCACGTTCGG TGCTGGGACC AAGCTGGAGC TGAAACGGGC TGTGCTGCA
GTACAAGGCG AGTGCAAGCC ACGACCCTGG TTCGACCTCG ACTTTGCCCG ACAACGACGT
98 H V P L T F G A G T K L E L K R A V A A
* * * *

421 CCAACTGTAT TCATCTTCCC ACCATCCAGT GAGCAATTGA AATCTGGAAC TGCCTCTGTT
GGTTGACATA AGTAGAAGGG TGGTAGGTCA CTCGTAACT TTAGACCTTG ACGGAGACAA
118 P T V F I F P P S S E Q L K S G T A S V

481 GTGTGCCTGC TGAATAACTT CTATCCCAGA GAGGCCAAAG TACAGTGGA GGTGGATAAC
CACACGGACG ACTTATTGAA GATAGGGTCT CTCCGGTTTC ATGTCACCTT CCACCTATTG
138 V C L L N N F Y P R E A K V Q W K V D N

541 GCCCTCCAAT CGGGTAACTC CCAGGAGAGT GTCACAGAGC AGGACAGCAA GGACAGCACC
CGGGAGGTTA GCCCATTTGAG GGTCTCTCA CAGTGTCTCG TCCTGTCTGT CCTGTCTGTG
158 A L Q S G N S Q E S V T E Q D S K D S T

601 TACAGCCTCA GCAGCACCTT GACGCTGAGC AAAGCAGACT ACGAGAAACA CAAAGTCTAC
ATGTCGGAGT CGTCGTGGGA CTGCGACTCG TTTCGTCTGA TGCTCTTTGT GTTTCAGATG
178 Y S L S S T L T L S K A D Y E K H K V Y

FIG. 27A

31 / 136

661 GCCTGCCAAG TCACCCATCA GGGCCTGAGC TCGCCCGTCA CAAAGAGCTT CAACAGGGGA
CGGACGCTTC AGTGGGTAGT CCCGGAATCG AGCGGCAGT GTTCTCGAA GTTGTCCTCCT
198 A C E V T H Q G L S S P V T K S F N R G

721 GAGTGTAA
CTCACAATT
218 E C O

FIG. 27B

32 / 136

1 ATGAAAAAGA ATATCGCATT TCTTCTTGCA TCTATGTTTCG TTTTCTCTAT TGCTACAAAC
 TACTTTTCTCT TATAGCGTAA AGAAGAACGT AGATACAAGC AAAAAAGATA ACGATGTTTG
 -23 M K K N I A F L L A S M F V F S I A T N

61 GCGTACGCTG AGATTACAGCT GCAGCAGTCT GGACCTGAGC TGATGAAGCC TGGGGCTTCA
 CGCATGCGAC TCTAAGTCGA CGTCGTCAGA CCTGGACTCG ACTACTTCGG ACCCCGAAGT
 -3 A Y A E I Q L Q Q S G P E L M K P G A S

121 GTGAAGATAT CCTGCAAGGC TTCTGGTTAT TCATTACAGTA GCCACTACAT GCACTGGGTG
 CACTTCTATA GGACGTTCCG AAGACCAATA AGTAAGTCAT CGGTGATGTA CGTGACCCAC
 18 V K I S C K A S G Y S F S S H Y M H W V
 * * * * *

CDR #1

181 AAGCAGAGCC ATGGAAAGAG CCTTGAGTGG ATTGGCTACA TTGATCCTTC CAATGGTGAA
 TTCGTCTCGG TACCTTTCTC GGAACCTACC TAACCGATGT AACTAGGAAG GTTACCACTT
 38 K Q S H G K S L E W I G Y I D P S N G E
 * * * * *

CDR #2

241 ACTACTTACA ACCAGAAATT CAAGGGCAAG GCCACATTGA CTGTAGACAC ATCTTCCAGC
 TGATGAATGT TGGTCTTTAA GTTCCCGTTC CGGTGTAAC TACATCTGTG TAGAAGGTCG
 58 T T Y N Q K F K G K A T L T V D T S S S
 * * * * *

301 ACAGCCAACG TGCATCTCAG CAGCCTGACA TCTGATGACT CTGCAGTCTA TTTCTGTGCA
 TGTCGGTTGC ACGTAGAGTC GTCGGACTGT AGACTACTGA GACGTCAGAT AAAGACACGT
 78 T A N V H L S S L T S D D S A V Y F C A

361 AGAGGGGACT ATAGATACAA CGGCGACTGG TTTTTCGATG TCTGGGGCGC AGGGACCACG
 TCTCCCCTGA TATCTATGTT GCCGCTGACC AAAAAGCTAC AGACCCCGCG TCCCTGGTGC
 98 R G D Y R Y N G D W F F D V W G A G T T
 * * * * *

CDR #3

421 GTCACCGTCT CCTCCGCCTC CACCAAGGGC CCATCGGTCT TCCCCCTGGC ACCCTCCTCC
 CAGTGGCAGA GGAGGCGGAG GTGGTTCCCG GGTAGCCAGA AGGGGGACCG TGGGAGGAGG
 118 V T V S S A S T K G P S V F P L A P S S

481 AAGAGCACCT CTGGGGGCAC AGCGGCCCTG GGCTGCCTGG TCAAGGACTA CTTCCCCGAA
 TTCTCGTGGA GACCCCGTG TCGCCGGGAC CCGACGGACC AGTTCCTGAT GAAGGGGCTT
 138 K S T S G G T A A L G C L V K D Y F P E

541 CCGGTGACGG TGTCGTGGAA CTCAGGCGCC CTGACCAGCG GCGTGCACAC CTTCCCGGCT
 GGCCACTGCC ACAGCACCTT GAGTCCGCGG GACTGGTCGC CGCACGTGTG GAAGGGCCGA
 158 P V T V S W N S G A L T S G V H T F P A

601 GTCCTACAGT CCTCAGGACT CTACTCCCTC AGCAGCGTGG TGACCGTGCC CTCCAGCAGC
 CAGGATGTCA GGAGTCCTGA GATGAGGGAG TCGTCGCACC ACTGGCACGG GAGGTCTGTCG
 178 V L Q S S G L Y S L S S V V T V P S S S

FIG. 28A

SUBSTITUTE SHEET (RULE 26)

33 / 136

661 TTGGGCACCC AGACCTACAT CTGCAACGTG AATCACAAGC CCAGCAACAC CAAGGTGGAC
AACCCGTGGG TCTGGATGTA GACGTTGCAC TTAGTGTTTCG GGTCTGTTGTG GTTCCACCTG
198 L G T Q T Y I C N V N H K P S N T K V D
721 AAGAAAGTTG AGCCCAATC TTGTGACAAA ACTCACACAT GA
TTCTTTCAAC TCGGGTTTAG AACACTGTTT TGAGTGTGTA CT
218 K K V E P K S C D K T H T O

FIG. 28B

34 / 136

Variable Light Chain Domain

	10	20	abcde	30	40
6G425	DIVMTQTPLSLPVSLGDAQASISCRSSQSLVHGIGNTYLHWYLQKPGQSPKLLIY				
	# # # # # # # # # #				
F(ab)-1	DIQMTQSPSSLSASVGDRVTITCRSSQSLVHGIGNTYLHWYQQKPGKAPKLLIY				
	# # # # # # # # # #				
humkI	DIQMTQSPSSLSASVGDRVTITCRASKTI-----SKYLAWYQQKPGKAPKLLIY				
	=====				
	+++++				
	L1				

	50	60	70	80	90	100
6G425	YKVSNRFSGVPDFRSDSGSGTDFTLRISRVEAEDLGLYFCSQSTHVPLTFGAGTKLELKR					
	# # # # # # # # # #					
F(ab)-1	YKVSNRFSGVPSRFSGSGSGTDFTLTISLQPEDFATYYCSQSTHVPLTFGQGTKVEIKR					
	# # # # # # # # # #					
humkI	YSGSTLESQVPSRFSGSGSGTDFTLTISLQPEDFATYYCQQHNEYPLTFGQGTKVEIKR					
	===					
	+++++					
	L2					

	50	60	70	80	90	100	110
6G425	EIQLQQSGPELMKPGASVKISCKASGYFSSHYMHVWKQSHGKSLEWI						
	# # # # # # # # # #						
F(ab)-1	EVQLVESGGGLVQPGGSLRLSCAASGYFSSHYMHVWRQAPGKGLEWV						
	# # # # # # # # # #						
humIII	EVQLVESGGGLVQPGGSLRLSCAASGFSTGHWMNWVRQAPGKGLEWV						
	=====						
	+++++						
	H1						

	50	a	70	80	abc	90	100	110
6G425	GYIDPSNGETTYNQKFKGKATLTVDTSSSTANVHLSSLTSDDSAVYFCAARGDYRYNGDWFFDVWGAGT							
	# # # # # # # # # #							
F(ab)-1	GYIDPSNGETTYNQKFKGRFTISRDNKNTLYLQMNSLRAEDTAVYYCAARGDYRYNGDWFFDVWGQGT							
	# # # # # # # # # #							
humIII	GMIHPSDSETRYADSVKGRFTISRDNKNTLYLQMNSLRAEDTAVYYCAARGIYFY-GTTYFDYWGQGT							
	=====							
	+++++							
	H2							

	50	a	70	80	abc	90	100	110
6G425	GYIDPSNGETTYNQKFKGKATLTVDTSSSTANVHLSSLTSDDSAVYFCAARGDYRYNGDWFFDVWGAGT							
	# # # # # # # # # #							
F(ab)-1	GYIDPSNGETTYNQKFKGRFTISRDNKNTLYLQMNSLRAEDTAVYYCAARGDYRYNGDWFFDVWGQGT							
	# # # # # # # # # #							
humIII	GMIHPSDSETRYADSVKGRFTISRDNKNTLYLQMNSLRAEDTAVYYCAARGIYFY-GTTYFDYWGQGT							
	=====							
	+++++							
	H3							

FIG. 29

35 / 136

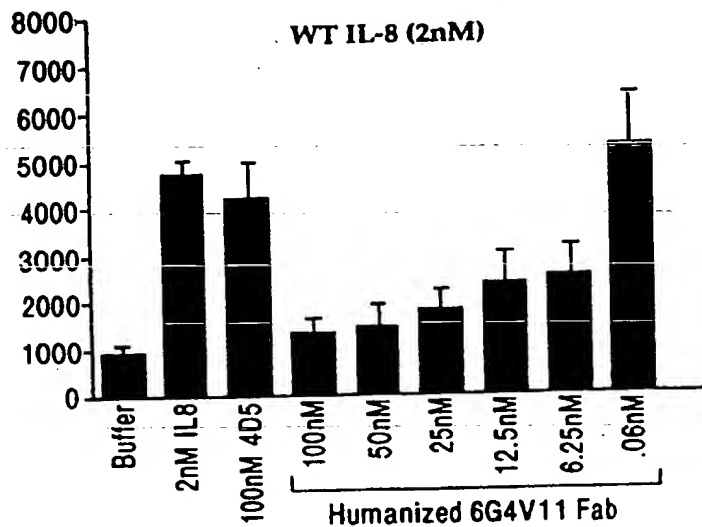


FIG. 30A

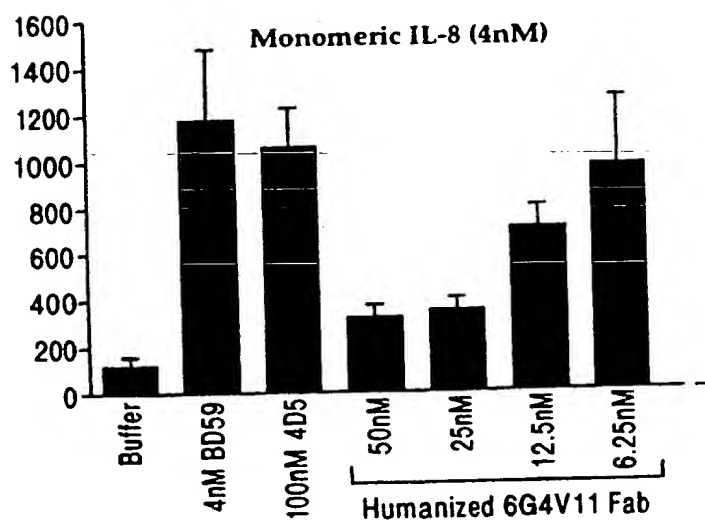
IC₅₀~12nM

FIG. 30B

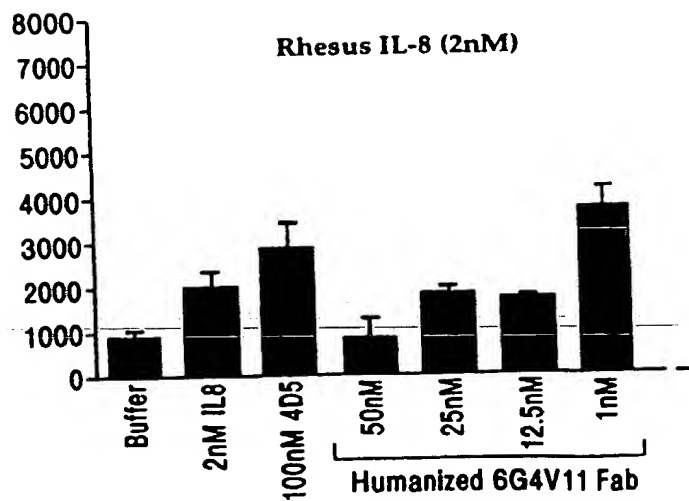
IC₅₀~15nM

FIG. 30C

IC₅₀~22nM

36 / 136

Amino Acid Sequence of the humanized anti-IL-8 6G4.2.5V11 Light Chain

MKKNIAFLLASMFVFSIATNAYADIQMTQSPSSLSASVGDRTTITCRSSQSLVHGIGNTY
 LHWYQQKPKAPKLLIYKVSNRFSGVPSRFSGSGGTDFTLTISSLQPEDFATYYCSQST
 HVPLTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDN
 ALQSGNSQESVTEQDSKDSSTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRG
 EC

Amino Acid Sequence of the humanized anti-IL-8 6G4.2.5V11 Heavy Chain

MKKNIAFLLASMFVFSIATNAYAEVQLVQSGGGLVQPGGSLRLSCAASGYSFSSHYMH
 WVRQAPGKGLEWVGVIDPSNGETTYNQFKGRFTLSRDNSKNTAYLQMNSLRAEDTAVYY
 CARGDYRYNGDWFDDVWGQTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYF
 PEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHHKPSNTK
 VDKKVEPKSCDKTHT

Amino Acid Sequence of the peptide linker and M13 Phage Coat (gene-III)

SGGSGSGDFDYEKMANANKGAMTENADENALQSDAKGKLDVATDYGAIDGFIGDVS
 GLANGGATGDFAGSSNSQMAQVGDDNSPLMNNFRQYLPQLPQSVCECRPFVFSAGKPY
 EFSIDCDKINLFRGVFAFLLYVATFMYVVFSTFANILLRNKES

FIG. 31A

37 / 136

1 ATGAAAAAGA ATATCGCATT TCTTCTTGCA TCTATGTTCG TTTTCTCTAT TGCTACAAAC
 TACTTTTTCT TATAGCGTAA AGAAGAACGT AGATACAAGC AAAAAAGATA ACGATGTTTG
 -23 M K K N I A F L L A S M F V F S I A T N
 61 GCATACGCTG ATATCCAGAT GACCCAGTCC CCGAGCTCCC TGTCCGCCTC TGTGGGCGAT
 CGTATGCGAC TATAGGTCTA CTGGGTCAGG GGCTCGAGGG ACAGGCGGAG ACACCCGCTA
 -3 A Y A D I Q M T Q S P S S L S A S V G D
 121 AGGGTCACCA TCACCTGCAG GTCAAGTCAA AGCTTAGTAC ATGGTATAGG TAACACGTAT
 TCCCAGTGGT AGTGGACGTC CAGTTCAGTT TCGAATCATG TACCATATCC ACGATGCATA
 18 R V T I T C R S S Q S L V H G I G N T Y
 181 TTACACTGGT ATCAACAGAA ACCAGGAAAA GCTCCGAAAC TACTGATTTA CAAAGTATCC
 AATGTGACCA TAGTTGTCTT TGGTCCTTTT CGAGGCTTTG ATGACTAAAT GTTTCATAGG
 38 L H W Y Q Q K P G K A P K L L I Y K V S
 241 AATCGATTCT CTGGAGTCCC TTCTCGCTTC TCTGGATCCG GTTCTGGGAC GGATTTCACT
 TTAGCTAAGA GACCTCAGGG AAGAGCGAAG AGACCTAGGC CAAGACCCTG CCTAAAGTGA
 58 N R F S G V P S R F S G S G S G T D F T
 301 CTGACCATCA GCAGTCTGCA GCCAGAAGAC TTCGCAACTT ATTACTGTTC ACAGAGTACT
 GACTGGTAGT CGTCAGACGT CGGTCTTCTG AAGCGTTGAA TAATGACAAG TGTCTCATGA
 78 L T I S S L Q P E D F A T Y Y C S Q S T
 361 CATGTCCCGC TCACGTTTGG ACAGGGTACC AAGGTGGAGA TCAAACGAAC TGTGGCTGCA
 GTACAGGGCG AGTGCAAACC TGTCCCATGG TTCCACCTCT AGTTTGCTTG ACACCGACGT
 98 H V P L T F G Q G T K V E I K R T V A A
 421 CCATCTGTCT TCATCTTCCC GCCATCTGAT GAGCAGTTGA AATCTGGAAC TGCTTCTGTT
 GGTAGACAGA AGTAGAAGGG CGGTAGACTA CTCGTCAACT TTAGACCTTG ACGAAGACAA
 118 P S V F I F P P S D E Q L K S G T A S V
 481 GTGTGCCTGC TGAATAACTT CTATCCCAGA GAGGCCAAAG TACAGTGGAA GGTGGATAAC
 CACACGGACG ACTTATTGAA GATAGGGTCT CTCCGGTTTC ATGTCACCTT CCACCTATTG
 138 V C L L N N F Y P R E A K V Q W K V D N
 541 GCCCTCCAAT CGGGTAACTC CCAGGAGAGT GTCACAGAGC AGGACAGCAA GGACAGCACC
 CGGGAGGTTA GCCCATTGAG GGTCTCTCTCA CAGTGTCTCG TCCTGTCTGTT CCTGTCTGTT
 158 A L Q S G N S Q E S V T E Q D S K D S T
 601 TACAGCCTCA GCAGCACCTT GACGCTGAGC AAAGCAGACT ACGAGAAACA CAAAGTCTAC
 ATGTCGGAGT CGTCGTGGGA CTGCGACTCG TTTCTGTCTGA TGCTCTTTGT GTTTCAGATG
 178 Y S L S S T L T L S K A D Y E K H K V Y
 661 GCCTGCGAAG TCACCCATCA GGGCCTGAGC TCGCCCGTCA CAAAGAGCTT CAACAGGGGA
 CGGACGCTTC AGTGGGTAGT CCCGGACTCG AGCGGGCAGT GTTCTCTGAA GTTGTCCCTT
 198 A C E V T H Q G L S S P V T K S F N R G
 721 GAGTGTTAAG CTGATCCTCT ACGCCGGAAG CATCGTGGCC CTAGTACGCA ACTAGTCGTA
 CTCACAATTC GACTAGGAGA TGCGGCCTGC GTAGCACCGG GATCATGCGT TGATCAGCAT
 218 E C O

FIG. 31B

38 / 136

Amino Acid Sequence of the humanized anti-IL-8 6G4.2.5V19 Light Chain

MKKNIAFLASMFVFSIATNAYADIQMTQSPSSLSASVGDRTITCRSSQSLVHGIGNTY
LHWYQQKPGKAPKLLIYKVSNRFSGVPSRFSGSGGTDFTLTISLQPEDFATYYCSQST
HVPLTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDN
ALQSGNSQESVTEQDSKDSITYSLSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRG
EC

Amino Acid Sequence of the humanized anti-IL-8 6G4.2.5V19 Heavy Chain

MKKNIAFLASMFVFSIATNAYAEVQLVESGGGLVQPGGSLRLSCAASGYSFSSHYMH
WVKQAPGKGLEWVGYYIDPSNGETTYNQKFKGRFTLSRDNSKNTAYLQMNSLRAEDTAVYY
CARGDYRYNGDWFFDVGQGTLVTVSSASTKGPSTFPLAPSSKSTSGGTAALGCLVKDYF
PEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHHKPSNTK
VDKKVEPKSCDKTHT

FIG. 31C

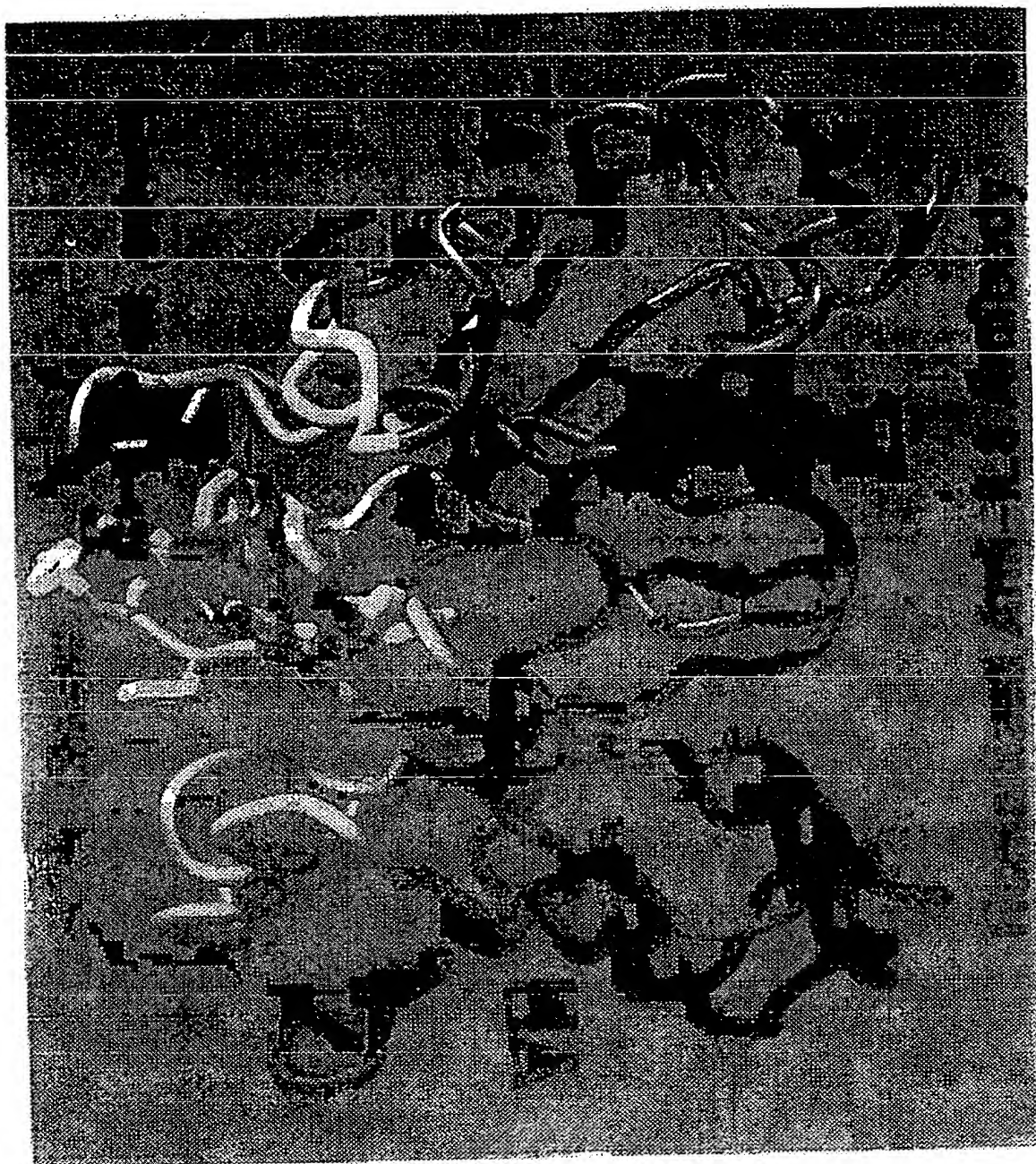


FIG.32

40/136

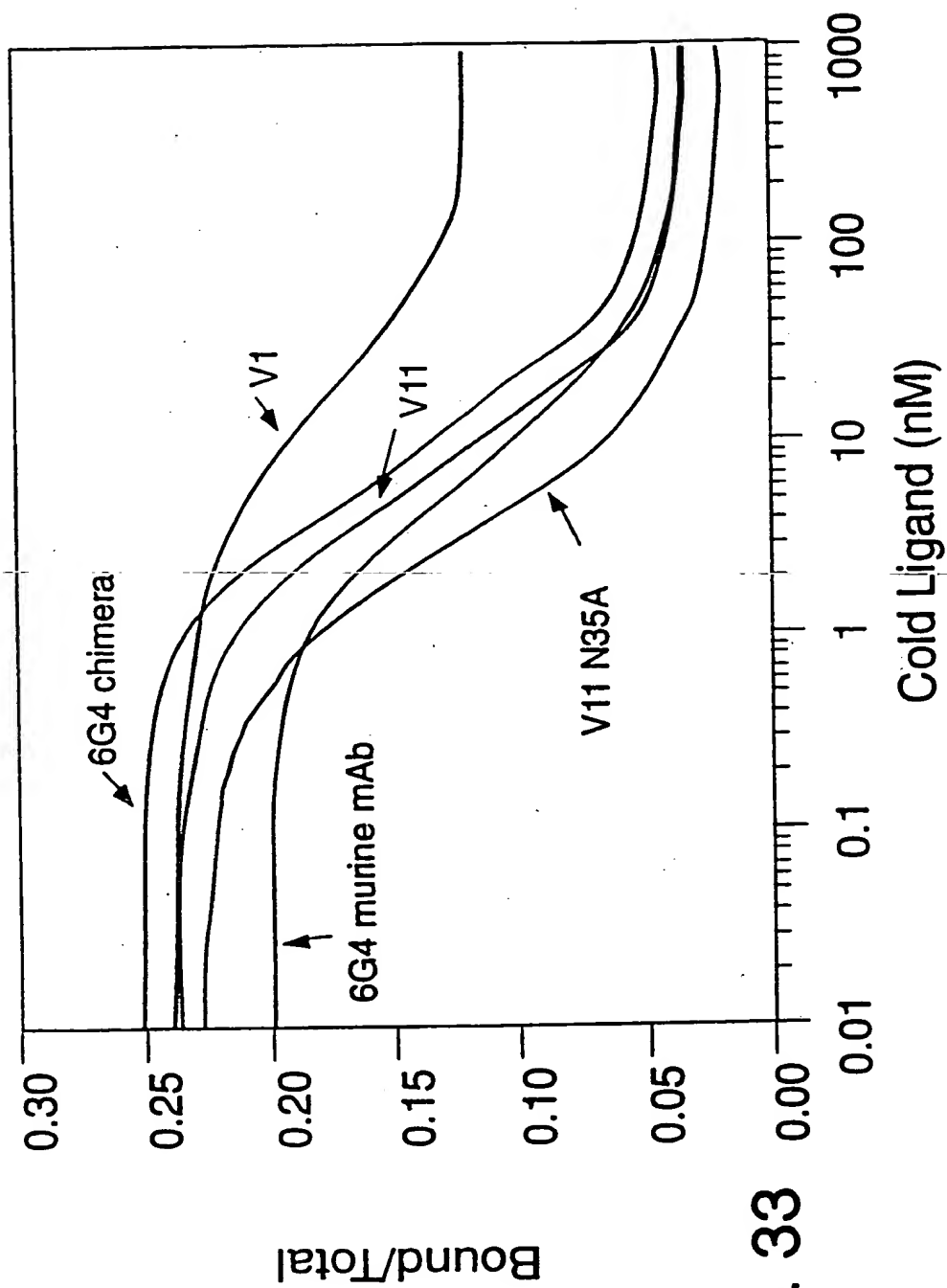
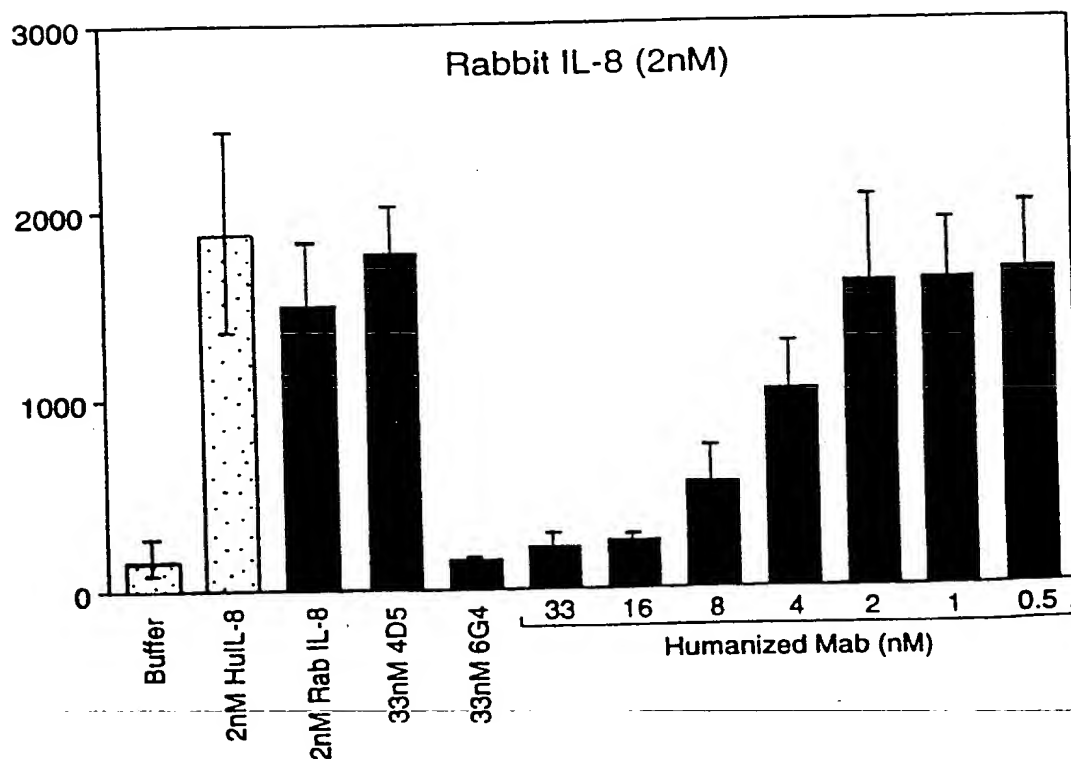
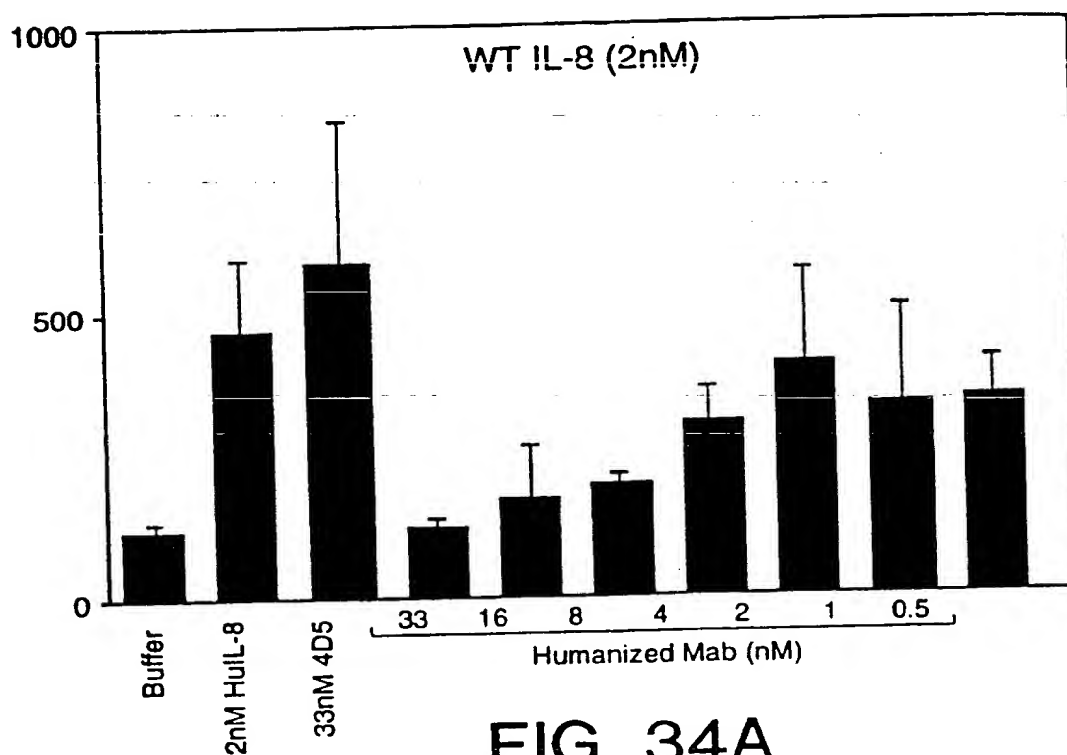


FIG. 33

41 / 136



42 / 136

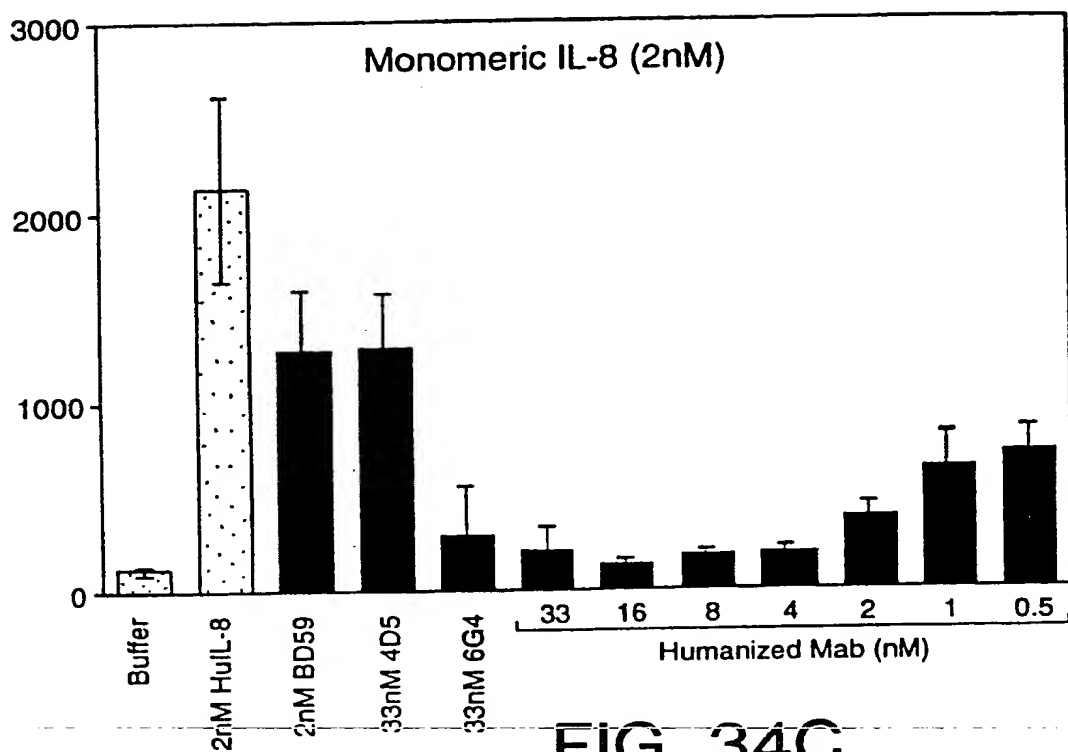


FIG. 34C

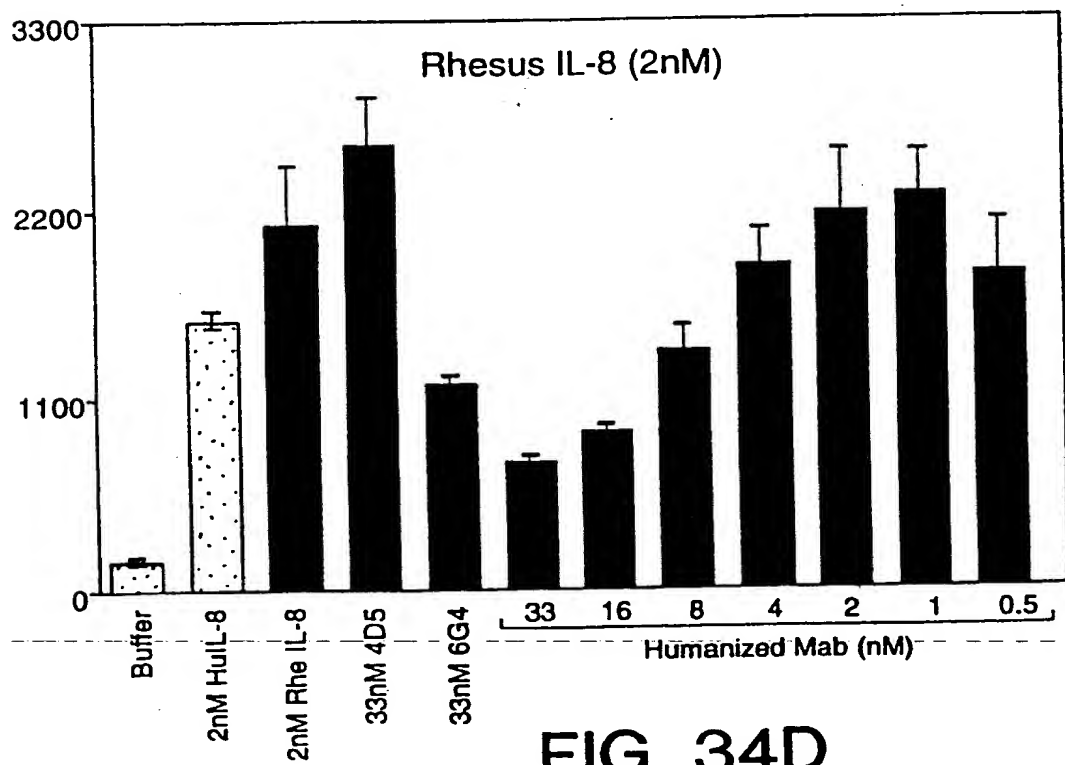


FIG. 34D

43 / 136

Amino Acid Sequence of the humanized anti-IL-8 6G4.2.5V11N35A Light Chain

MKKNIAFLASMFVFSIATNAYADIQMTQSPSSLSASVGDRVTITCRSSQSLVHGIGATY
LHWYQQKPGKAPKLLIYKVSNNRFSGVPSRFSGSGGTDFLTITSSLPEDFATYYCSQST
HVPLTFGQGTKEIKRTVAAPSVFIFPPSDEQLKSGTASVVCCLNNFYPREAKVQWKVDN
ALQSGNSQESVTEQDSKSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRG
EC

Amino Acid Sequence of the humanized anti-IL-8 6G4.2.5V11N35A Heavy Chain

MKKNIAFLASMFVFSIATNAYAEVQLVQSGGGLVQPGGSLRLSCAASGYSFSSHYMH
WVRQAPGKGLEWVGYIDPSNGETTYNQKFKGRFTLSRDNSKNTAYLQMNSLRAEDTAVYY
CARGDYRYNGDWFDFVWGQGLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYF
PEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTK
VDKKVEPKSCDKTHT

Amino Acid Sequence of the putative Pepsin Cleavage Site and GCN4 Leucine Zipper

CPPCPAP~~ELL~~GGRMKQLEDKVEELL~~SKNYH~~LENEVARLKKLVGER

FIG. 35

44 / 136

```

1 ATGAAAAAGA ATATCGCATT TCTTCTTGCA TCTATGTTTCG TTTTCTTCTAT TGCTACAAAC
  TACTTTTTTCT TATAGCGTAA AGAAGAACGT AGATACAAGC AAAAAAGATA ACGATGTTTG
-23 M K K N I A F L L A S M F V F S I A T N

61 GCATACGCTG ATATCCAGAT GACCCAGTCC CCGAGCTCCC TGTCCGCCTC TGTGGGCGAT
  CGTATGCGAC TATAGGTCTA CTGGGTCAGG GGCTCGAGGG ACAGGCGGAG ACACCCGCTA
-3 A Y A D I Q M T Q S P S S L S A S V G D

121 AGGGTCACCA TCACCTGCAG GTCAAGTCAA AGCTTAGTAC ATGGTATAGG TGCTACGTAT
  TCCCAGTGGT AGTGGACGTC CAGTTCAGTT TCGAATCATG TACCATATCC ACGATGCATA
18 R V T I T C R S S O S L V H G I G A T Y

181 TTACACTGGT ATCAACAGAA ACCAGGAAAA GCTCCGAAAC TACTGATTTA CAAAGTATCC
  AATGTGACCA TAGTTGTCTT TGGTCCTTTT CGAGGCTTTG ATGACTAAAT GTTTCATAGG
38 L H W Y Q Q K P G K A P K L L I Y K V S

241 AATCGATTCT CTGGAGTCCC TTCTCGCTTC TCTGGATCCG GTTCTGGGAC GGATTTCACT
  TTAGCTAAGA GACCTCAGGG AAGAGCGAAG AGACCTAGGC CAAGACCCTG CCTAAAGTGA
58 N R F S G V P S R F S G S G S G T D F T

301 CTGACCATCA GCAGTCTGCA GCCAGAAGAC TTCGCAACTT ATTACTGTTC ACAGAGTACT
  GACTGGTAGT CGTCAGACGT CGGTCTTCTG AAGCGTTGAA TAATGACAAG TGTCTCATGA
78 L T I S S L Q P E D F A T Y Y C S Q S T

361 CATGTCCCGC TCACGTTTGG ACAGGGTACC AAGGTGGAGA TCAAACGAAC TGTGGCTGCA
  GTACAGGGCG AGTGCAAACC TGTCCCATGG TTCCACCTCT AGTTTGCTTG ACACCGACGT
98 H V P L T F G Q G T K V E I K R T V A A

421 CCATCTGTCT TCATCTTCCC GCCATCTGAT GAGCAGTTGA AATCTGGAAC TGCTTCTGTT
  GGTAGACAGA AGTAGAAGGG CGGTAGACTA CTCGTCAACT TTAGACCTTG ACGAAGACAA
118 P S V F I F P P S D E Q L K S G T A S V

481 GTGTGCCTGC TGAATAACTT CTATCCCAGA GAGGCCAAAG TACAGTGGAA GGTGGATAAC
  CACACGGACG ACTTATTGAA GATAGGGTCT CTCCGGTTTC ATGTCACCTT CCACCTATTG
138 V C L L N N F Y P R E A K V Q W K V D N

541 GCCCTCCAAT CGGGTAACTC CCAGGAGAGT GTCACAGAGC AGGACAGCAA GGACAGCACC
  CGGGAGGTTA GCCCATTTGAG GGTCTCTCA CAGTGTCTCG TCCTGTCTGT CCTGTCTGTG
158 A L Q S G N S Q E S V T E Q D S K D S T

601 TACAGCCTCA GCAGCACCTT GACGCTGAGC AAAGCAGACT ACGAGAAACA CAAAGTCTAC
  ATGTCGGAGT CGTCGTGGGA CTGCGACTCG TTTCGTCTGA TGCTCTTTGT GTTTCAGATG
178 Y S L S S T L T L S K A D Y E K H K V Y

661 GCCTGCGAAG TCACCCATCA GGGCCTGAGC TCGCCCGTCA CAAAGAGCTT CAACAGGGGA
  CGGACGCTTC AGTGGGTAGT CCCGACTCG AGCGGGCAGT GTTCTCTGAA GTTGTCCCCT
198 A C E V T H Q G L S S P V T K S F N R G

721 GAGTGTTAAG CTGATCCTCT ACGCCGGACG CATCGTGGCC CTAGTACGCA ACTAGTCGTA
  CTCACAATTC GACTAGGAGA TCGGCCTGC GTAGCACCAG GATCATGCGT TGATCAGCAT
218 E C O

```

FIG. 36

45 / 136

781 AAAAGGGTAT CTAGAGGTTG AGGTGATTTT ATGAAAAAGA ATATCGCATT TCTTCTTGCA
 TTTTCCCAT A GATCTCCAAC TCCACTAAAA TACTTTTTCT TATAGCGTAA AGAAGAACGT
 -1 M K K N I A F L L A

841 TCTATGTTCTG TTTTCTCTAT TGCTACAAAC GCGTACGCTG AGGTTTCAGCT AGTGCAGTCT
 AGATACAAGC AAAAAAGATA ACGATGTTTG CGCATGCGAC TCCAAGTCGA TCACGTCAGA
 -11 S M F V F S I A T N A Y A E V Q L V Q S

901 GCGGGTGGCC TGGTGCAGCC AGGGGGCTCA CTCCGTTTGT CCTGTGCAGC TTCTGGCTAC
 CCGCCACCGG ACCACGTCGG TCCCCCGAGT GAGGCAAACA GGACACGTCG AAGACCGATG
 8 G G G L V Q P G G S L R L S C A A S G Y

961 TCCTTCTCGA GTCACTATAT GCACTGGGTC CGTCAGGCCC CGGGTAAGGG CCTGGAATGG
 AGGAAGAGCT CAGTGATATA CGTGACCCAG GCAGTCCGGG GCCCATTTCC GGACCTTACC
 28 S F S S H Y M H W V R Q A P G K G L E W

1021 GTTGGATATA TTGATCCTTC CAATGGTGAA ACTACGTATA ATCAAAAGTT CAAGGGCCGT
 CAACCTATAT AACTAGGAAG GTTACCACTT TGATGCATAT TAGTTTTCAA GTTCCCGGCA
 48 V G Y I D P S N G E T T Y N O K F K G R

1081 TTCACCTTAT CTCGCGACAA CTCCAAAAAC ACAGCATACC TGCAGATGAA CAGCCTGCGT
 AAGTGAAATA GAGCGCTGTT GAGGTTTTTG TGTCGTATGG ACGTCTACTT GTCGGACGCA
 68 F T L S R D N S K N T A Y L Q M N S L R

1141 GCTGAGGACA CTGCCGTCTA TTA CTGTGCA AGAGGGGATT ATCGCTACAA TGGTGA CTGG
 CGACTCCTGT GACGGCAGAT AATGACACGT TCTCCCCTAA TAGCGATGTT ACCACTGACC
 88 A E D T A V Y Y C A R G D Y R Y N G D W

1201 TTCTTCGACG TCTGGGGTCA AGGAACCCTG GTCACCGTCT CCTCGGCCTC CACCAAGGGC
 AAGAAGCTGC AGACCCCACT TCCTTGGGAC CAGTGGCAGA GGAGCCGGAG GTGGTTCCCG
 108 F F D V W G Q G T L V T V S S A S T K G

1261 CCATCGGTCT TCCCCCTGGC ACCCTCCTCC AAGAGCACCT CTGGGGGCAC AGCGGCCCTG
 GGTAGCCAGA AGGGGGACCG TGGGAGGAGG TTCTCGTGGA GACCCCGTG TCGCCGGGAC
 128 P S V F P L A P S S K S T S G G T A A L

1321 GGCTGCCTGG TCAAGGACTA CTTCCCCGAA CCGGTGACGG TGTCGTGGAA CTCAGGCGCC
 CCGACGGACC AGTTCCTGAT GAAGGGGCTT GGCCACTGCC ACAGCACCTT GAGTCCGCGG
 148 G C L V K D Y F P E P V T V S W N S G A

1381 CTGACCAGCG GCGTGCACAC CTTCCCCGGCT GTCCTACAGT CCTCAGGACT CTACTCCCTC
 GACTGGTTCG CGCACGTGTG GAAGGGCCGA CAGGATGTCA GGAGTCCTGA GATGAGGGAG
 168 L T S G V H T F P A V L Q S S G L Y S L

1441 AGCAGCGTGG TGACCGTGCC CTCCAGCAGC TTGGGCACCC AGACCTACAT CTGCAACGTG
 TCGTCGCACC ACTGGCACGG GAGGTCGTCG AACCCGTGGG TCTGGATGTA GACGTTGCAC
 188 S S V V T V P S S S L G T Q T Y I C N V

1501 AATCACAAGC CCAGCAACAC CAAGGTCGAC AAGAAAGTTG AGCCCAAATC TTGTGACAAA
 TTAGTGTTCT GGTGCTGTG GTTCCAGCTG TTCTTTCAAC TCGGGTTTAG AACACTGTTT
 208 N H K P S N T K V D K K V E P K S C D K

1561 ACTCACACAT GCCCGCCGTG CCCAGCACCA GAACTGCTGG GCGGCCGCAT GAAACAGCTA
 TGAGTGTTGA CGGGCGGCAC GGGTCGTGGT CTTGACGACC CGCCGGCGTA CTTTGTGCGAT
 228 T H T C P P C P A P E L L G G R M K Q L

FIG. 37A

46 / 136

1621 GAGGACAAGG TCGAAGAGCT ACTCTCCAAG AACTACCACC TAGAGAATGA AGTGGCAAGA
CTCCTGTTCC AGCTTCTCGA TGAGAGGTTC TTGATGGTGG ATCTCTTACT TCACCGTTCT
248 E D K V E E L L S K N Y H L E N E V A R

1681 CTCAAAAAGC TTGTCGGGGA GCGCTAA
GAGTTTTTCG AACAGCCCCT CGCGATT
268 L K K L V G E R O

FIG. 37B

47/136

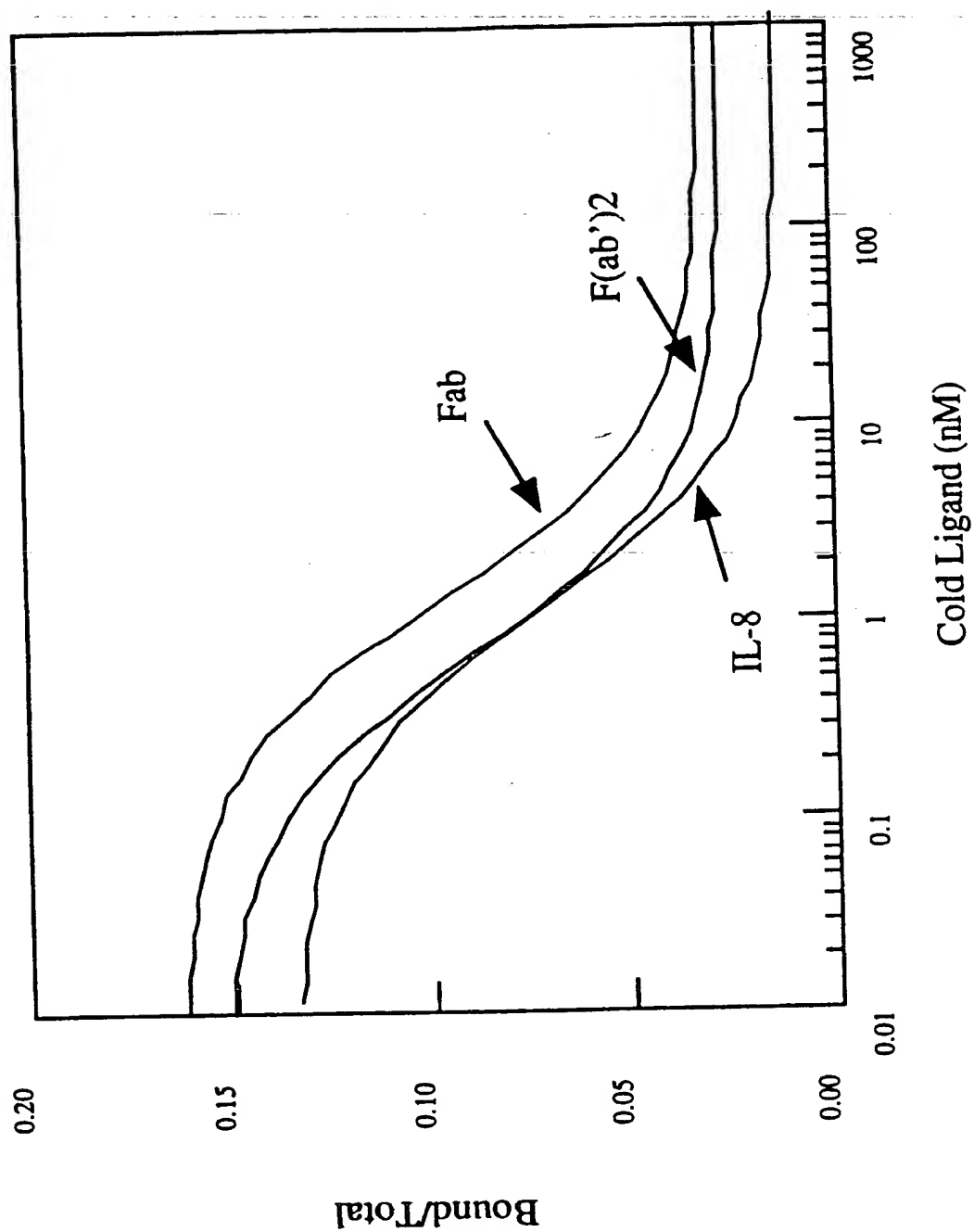


FIG. 38

48/136

FIG. 39

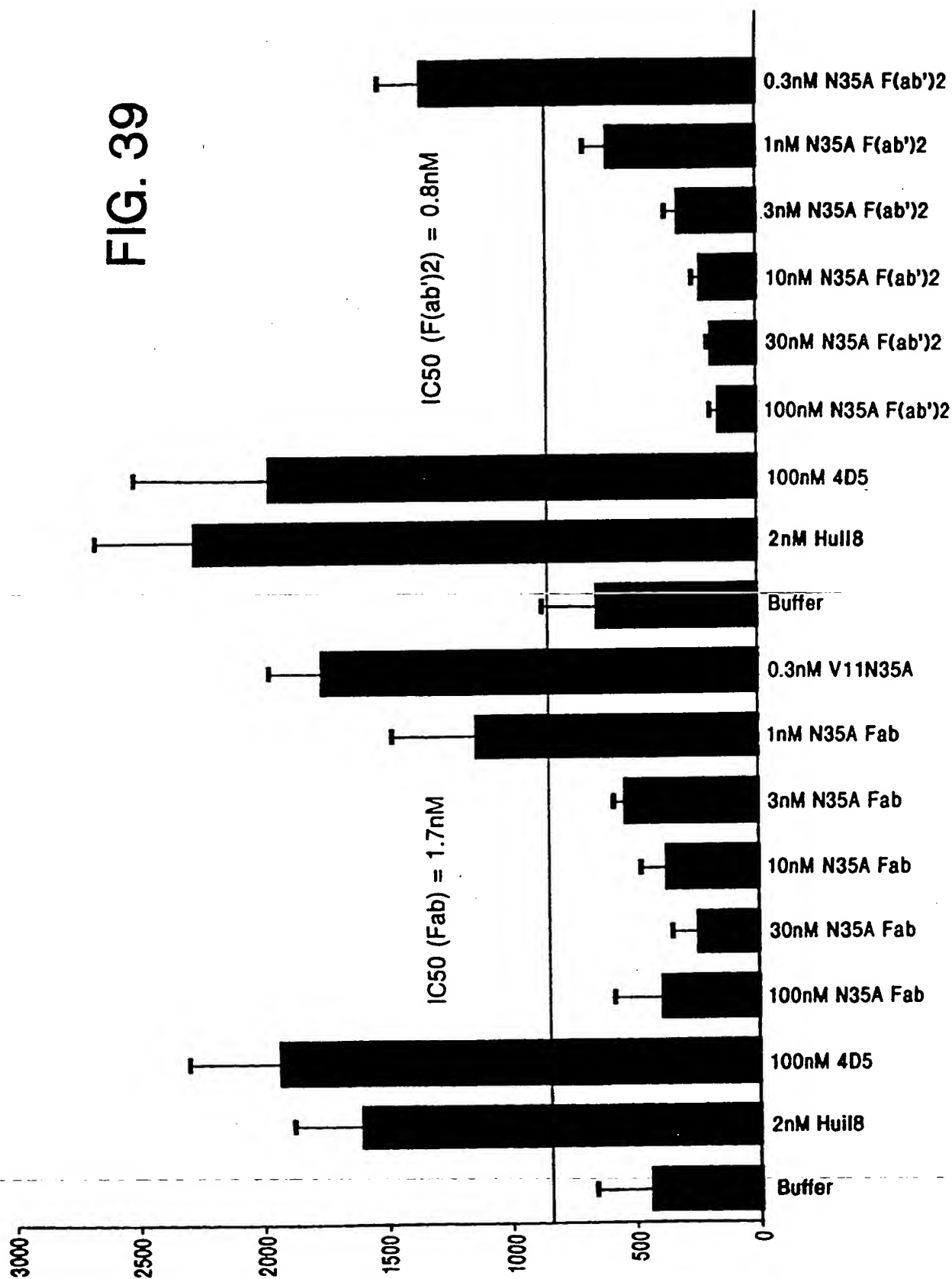
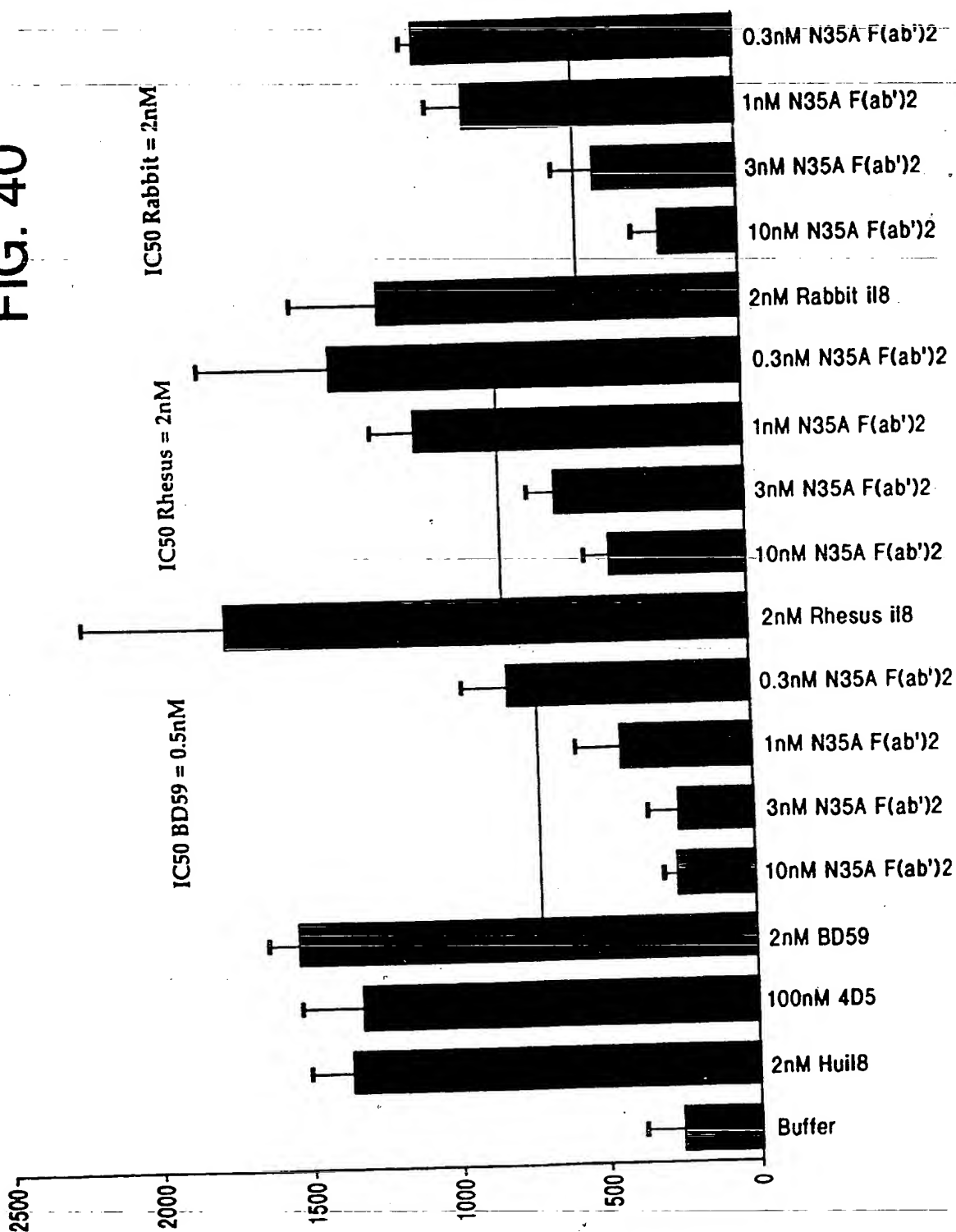


FIG. 40



Restriction Enzyme	Sequence	Restriction Enzyme	Sequence
1	GAATTC	1	GAATTC
2	GAATTC	2	GAATTC
3	GAATTC	3	GAATTC
4	GAATTC	4	GAATTC
5	GAATTC	5	GAATTC
6	GAATTC	6	GAATTC
7	GAATTC	7	GAATTC
8	GAATTC	8	GAATTC
9	GAATTC	9	GAATTC
10	GAATTC	10	GAATTC
11	GAATTC	11	GAATTC
12	GAATTC	12	GAATTC
13	GAATTC	13	GAATTC
14	GAATTC	14	GAATTC
15	GAATTC	15	GAATTC
16	GAATTC	16	GAATTC
17	GAATTC	17	GAATTC
18	GAATTC	18	GAATTC
19	GAATTC	19	GAATTC
20	GAATTC	20	GAATTC
21	GAATTC	21	GAATTC
22	GAATTC	22	GAATTC
23	GAATTC	23	GAATTC
24	GAATTC	24	GAATTC
25	GAATTC	25	GAATTC
26	GAATTC	26	GAATTC
27	GAATTC	27	GAATTC
28	GAATTC	28	GAATTC
29	GAATTC	29	GAATTC
30	GAATTC	30	GAATTC
31	GAATTC	31	GAATTC
32	GAATTC	32	GAATTC
33	GAATTC	33	GAATTC
34	GAATTC	34	GAATTC
35	GAATTC	35	GAATTC
36	GAATTC	36	GAATTC
37	GAATTC	37	GAATTC
38	GAATTC	38	GAATTC
39	GAATTC	39	GAATTC
40	GAATTC	40	GAATTC
41	GAATTC	41	GAATTC
42	GAATTC	42	GAATTC
43	GAATTC	43	GAATTC
44	GAATTC	44	GAATTC
45	GAATTC	45	GAATTC
46	GAATTC	46	GAATTC
47	GAATTC	47	GAATTC
48	GAATTC	48	GAATTC
49	GAATTC	49	GAATTC
50	GAATTC	50	GAATTC
51	GAATTC	51	GAATTC
52	GAATTC	52	GAATTC
53	GAATTC	53	GAATTC
54	GAATTC	54	GAATTC
55	GAATTC	55	GAATTC
56	GAATTC	56	GAATTC
57	GAATTC	57	GAATTC
58	GAATTC	58	GAATTC
59	GAATTC	59	GAATTC
60	GAATTC	60	GAATTC
61	GAATTC	61	GAATTC
62	GAATTC	62	GAATTC
63	GAATTC	63	GAATTC
64	GAATTC	64	GAATTC
65	GAATTC	65	GAATTC
66	GAATTC	66	GAATTC
67	GAATTC	67	GAATTC
68	GAATTC	68	GAATTC
69	GAATTC	69	GAATTC
70	GAATTC	70	GAATTC
71	GAATTC	71	GAATTC
72	GAATTC	72	GAATTC
73	GAATTC	73	GAATTC
74	GAATTC	74	GAATTC
75	GAATTC	75	GAATTC
76	GAATTC	76	GAATTC
77	GAATTC	77	GAATTC
78	GAATTC	78	GAATTC
79	GAATTC	79	GAATTC
80	GAATTC	80	GAATTC
81	GAATTC	81	GAATTC
82	GAATTC	82	GAATTC
83	GAATTC	83	GAATTC
84	GAATTC	84	GAATTC
85	GAATTC	85	GAATTC
86	GAATTC	86	GAATTC
87	GAATTC	87	GAATTC
88	GAATTC	88	GAATTC
89	GAATTC	89	GAATTC
90	GAATTC	90	GAATTC
91	GAATTC	91	GAATTC
92	GAATTC	92	GAATTC
93	GAATTC	93	GAATTC
94	GAATTC	94	GAATTC
95	GAATTC	95	GAATTC
96	GAATTC	96	GAATTC
97	GAATTC	97	GAATTC
98	GAATTC	98	GAATTC
99	GAATTC	99	GAATTC

FIG. 41A

```

scrFI
ncII
mspl
hpall
dsav
xmaI/pspAI
smaI
scrFI
ncII
dsav
cauII
bsaJI
avaI
sau3AI taqI
rsaI
msp6I
nlaIV nlaIV paer7I
kpnI cauII dpuII(dam-)
hgICl bstYI/xhoII
bani bsaJI alwI[dam-]
asp718 bamHI avaI
acc65I alwI[dam-] mnlI mnlI
TCGGTACCG GGGATCCTCT CGAGGTTGAG GTGATTITAT GAAAAAGAAT ATCGCATTC TTCTGCATC TATGTTCGTT TTTTCTAATG CTACAACGC
AGCCATGGGC CCTAGGAGA GTCCAAC TC CACTAAATA CTTTTCTTA TAGCGTAAAG AAGACGTAG ATACAAGCAA AAAAGATAAC GATGTTTCGG
M K K N I A F L L A S M F V F S I A T N A
a mutation was found that inactivated the mlul site. The penultimate nucleotide was changed fr G to ^
sstI
sacI
hgIJII
hgIAI/aspHI
ecII36II
bsp1286
bsiHKAI
bsmFI bmyI
bsrI avai alul
tthIII/aspI baniI
ecoRV
ATACGCTGAT ATCAGATGA CCCAGTCCCC GAGCTCCCTG TCCGCCCTCTG TGGCGGATAG GGTCACCATC ACCTGCAAGT CAAGTCAAAG CTAGTACAT
TATGCGACTA TAGTCTACT GGGTCAGGGG CTCGAGGGAC AGGCGGAGAC ACCGCTATC CCAGTGGTAG TGGACGTCCA GTTCAGTTTC GAATCATGTA
Y A D I Q M T Q S P S S L S A S V G D R V T I T C R S S Q S L V H

```


[illegible]

FIG. 41D

54 / 136

rmaI
 maeI
 bfaI
 xbaI mnlI mnlI hphI
 rsaI speI
 csp6I
 1201 AGTACGCAAC TAGTCGTAAA AAGGTATCT AGAGGTGAG GTGATTTTAT GAAGAAGAAT ATGCAATTTC TTCTTGCATC TATGTTTCGT TTTTCTATTG
 TCATGGGTTG ATCAGCATTT TTCCCATAGA TCTCCAATC TCTCCAACTC CACTAAATA CTTTCTCTTA TAGCGTAAAG AGAACGTAG ATACAAGCAA AAAAGATAAC
 M K E N I A F L L A S M F V F S I A

-23

scrFI
 mvaI
 ecorII
 dsav
 scrFI
 mvaI fnu4HI
 ecorII
 dsav bstNI hgiII
 bstNI bsoFI bsp1286
 apyI(dcm+) bsaJI bmyI
 haeIII/palI apyI(dcm+)
 acII haeI bbyI banII
 aluI
 alwNI(dcm-)
 fnu4HI
 bsoFI
 bbyI
 1301 CTACAAACGC GTACGCTGAG GTTCAGCTAG TGCAGTCTGG CGGTGGCCTG GTGCAGCCAG GGGCTCACT CCGTTGTCC TGTGCAGCTT CTGGCTACTC
 GATCTTGG CATGCGACTC CAAGTCGATC ACCTCAGACC GCCACCGGAC CAGTCTGGTC CCCCGAGTGA GGCAACAGG ACACGTCGAA GACCGATGAG
 -5 T N A Y A E V Q L V Q S G G G L V Q P G G S L R L S C A A S G Y S

scrFI
 nciI
 mspI
 hpaII
 dsav
 cauII
 bslI
 xmaI/pspAI
 smaI
 scrFI
 nciI
 dsav
 cauII
 bslI

scrFI
 mvaI
 ecorII

FIG. 41E

[illegible]

FIG. 41F

59 / 136

```

hinPI      hinPI      hinPI      hinPI      hinPI      hinPI      hinPI      hinPI      hinPI      hinPI
hhaI/cfoI  hhaI/cfoI  hhaI/cfoI  hhaI/cfoI  hhaI/cfoI  hhaI/cfoI  hhaI/cfoI  hhaI/cfoI  hhaI/cfoI  hhaI/cfoI
nlaIV      nlaIV      nlaIV      nlaIV      nlaIV      nlaIV      nlaIV      nlaIV      nlaIV      nlaIV
nari       nari       nari       nari       nari       nari       nari       nari       nari       nari
kasi       kasi       kasi       kasi       kasi       kasi       kasi       kasi       kasi       kasi
hinII/acyI hinII/acyI hinII/acyI hinII/acyI hinII/acyI hinII/acyI hinII/acyI hinII/acyI hinII/acyI hinII/acyI
hgiCI      hgiCI      hgiCI      hgiCI      hgiCI      hgiCI      hgiCI      hgiCI      hgiCI      hgiCI
haeII      haeII      haeII      haeII      haeII      haeII      haeII      haeII      haeII      haeII
bani       bani       bani       bani       bani       bani       bani       bani       bani       bani
ahaII/bsaHI ahaII/bsaHI ahaII/bsaHI ahaII/bsaHI ahaII/bsaHI ahaII/bsaHI ahaII/bsaHI ahaII/bsaHI ahaII/bsaHI ahaII/bsaHI
cac8I      cac8I      cac8I      cac8I      cac8I      cac8I      cac8I      cac8I      cac8I      cac8I
acII       acII       acII       acII       acII       acII       acII       acII       acII       acII
cfrI       cfrI       cfrI       cfrI       cfrI       cfrI       cfrI       cfrI       cfrI       cfrI
sfanI      sfanI      sfanI      sfanI      sfanI      sfanI      sfanI      sfanI      sfanI      sfanI
cfr101/bsrFI bsrFI bsrFI bsrFI bsrFI bsrFI bsrFI bsrFI bsrFI bsrFI
caccGGCGCA TCACCGCGC CACAGGTGCG GTTGCTGGCG CCTATATCGC CGACATCACC GATGGGGAAG ATCGGGCTCG CCACCTCGGG CTCATGAGCG
CACC GGCCCT AGTGGCGCG GTGTCCACGC CAACGACCGC GGATATAGCG GCTGTATGCG CTACCCCTTC TAGCCCGAGC GGTGAAGCCC GAGTACTCGC
2601
2701 CTTGTTTCGG CTGGGTATG GTGGCAGGCC CCGTGCGCCG GGGACTGTTG GCGCCATCT CCTGCACGC ACCATTCTT GCGCGGCGG TGCTCAACGG
GAACAAGCC GCACCCATAC CACCGTCCG GGCACCGGCC CCTGACAAAC CCGCGGTAGA GGAACGTGG TGGTAAGGAA CGCGCGCGCC ACGAGTTGCC
2801 CCTCAACCTA CTACTGGGT GCTTCCTAAT GCAGGAGTCG CATACGGGAG AGGTCGTCC GATGCCCTTG AGAGCCTTCA ACCAGTCAG CTCCTTCGG
GGAGTTGGAT GATGACCCGA CGAAGGATTA GTCCTCACC GTCTCTCAGC GTATTCCCTC TCGCAGCAGG CTACGGGAAC TCTCGAAGT TGGGTCAGTC GAGGAAGGCC

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FIG. 41J

60 / 136

2901 TGGGCGGGG GCATGACTAT CGTCGCGCGCA CTTATGACTG TCTTCTTTAT CATGCNACTC GTAGGACAGG TCCCGGCAGC GCTCTGGGTC ATTTTCGGCG
 ACCCGCGCCC CGTACTGATA GCAGCGGCGT GAATACTGAC AGAAGAATA GTACGTTGAG CATCTGTCC ACGGCGCTCG CGAGACCCAG TAAAGCCGC
 3001 AGGACCGCTT TCGCTGGAGC GCGACGATGA TCGGCGCTGC GCTTGGGTA TCGGAACTT TGCACGCCCT CCCTCAAGCC TCGTCACTG GTCCCGCCAG
 TCCTGGCGAA AGCGACCTCG CGCTGCTACT AGCCGGACAG CGAACGCCAT AAGCCTTAGA ACGTCGGGA CGAGTTCCG AAGCAGTGAC CAGGCGGCTG
 3101 CAACGTTT GCGGAGAAGC AGGCCATTAT CGCCGGCATG GCGGCGGACG CGCTGGGCTA CGTCTTGCTG GCGTTCGGA CGCAGGCTG GATGGCTTC
 GTTTCGAAG CCGCTCTTCG TCCGGTAATA GCGGCGGTAC CGCGGCTGC GCGACCCGAT GCAGAACGAC CGCAAGCGCT GCGTCCGAC CTACCGAAG

FIG. 41K

[illegible]

FIG. 41L

62 / 136

33501 CTAACGGATT CACCACATCCA AGAATTGGAG CCATCAATT CTTCGGGAGA ACTGTGAATG CGCAACCAA CCCTTGGCAG AACATATCCA TCAGCTCCGC
GATTGCTAA GTGGTGAGGT TCTTAACCTC GGTTAGTTAA GAACGCTCT TGACACTTAC GCGTTTGGTT GGAACCGTC TTGTAATAGT AGCGACGGC

3601 CATCTCCAG AGCCGACGC GGGCATCTC GGGCAGCGT GGTCTCTGC CACGGTGGC CATGATCGT CTCCTGCTCGT TGAGGACCG GTAGGCTGG
GTAGAGGTG TCGGCGTGG CCGCTAGAG CCGCTCGAA CCCAGGACG GTGCCACGC GTACTAGCAC GAGGACAGCA ACTCTGGG CGATCCGACC

FIG. 41M

63 / 136

```

sau3AI
mboI/ndelI{dam-}
mamI{dam-}
dpuI{dam+}
dpuI{dam-}
bstYI/xhoII
alwI{dam-}
mspI
hpaII
mroI bsalI{dam-}
bspHII
bspEI{dam-}
bsaWI sfaNI
accIII{dam-} foki cac8I
TTATGTTCCG GATCTGCATC GCAGGATGCT GCTGGCTACC
3801 TGGTCTTCGG TTTCGGTGTT TCGTAAAGTC TGGAAACGG GAAGTCAGCG CCTGACACCA
ACCAAGAGCC AAAGGCACAA AGCATTTTCAG ACCTTTGCGC CTTCACTCGC GGGACGTGGT AATACNAGGC CTAGACGTAG CGTCTACGA CGACCCGATGG
accII
bsmFI foki
sau96I sfaNI
nlaIV acII
avaII fnu4HI
asuI bsoFI
bsrI
3901 CTGTGGAACA CCTACATCTG TATTAACGAA GCGCTGGCAT TGACCTTGAG TGATTTTCT CTGCTCCGC CGCATCCATA CCGCCAGTTG TTTACCCCTCA
GACACCTTGT GGATGTAGAC ATAATTGCTT CCGGACCGTA ACTGGGACTC ACTAAAAGA GACCAGGGCG GCTAGGTAT GCGGTCTAC AAATGGGAGT
nspI
scrFI
ncII
mspI
bsrI
bsII
maeII
psp1406I maelII nspHI
4001 CAACGTTCCA GTAACCGGC ATGTTTCATCA TCAGTAACCC GTATCGTGAG CATCCTCTCT CGTTTCATCG GTATCATTAC CCCCATGAAC AGAATTCCC
GTTGCAAGGT CATGGCCCG TACAGTAGT AGTCATTGGG CATAGCACTC GTAGGAGAGA GCAAAGTAGC CATAGTAATG GGGTACTTG TCTTTAAGGG
nlaIII apoI bslI

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FIG. 41N

64 / 136

[illegible]

FIG. 410

SUBSTITUTE SHEET (RULE 26)

66 / 136

Position	Sequence	Restriction Enzyme
4801	GAACCCGAC AGGACTATAA AGATACGAG CGTTTCCCC TGGAGCTCC CTCGTGGCT CTCTGTTC GACCTGGCG CTTACCGGAT ACCTGTCCGC CTTTGGCTG TCTGTATTT TCTATGGTCC GCAAGGGGG ACCTTCGAG GAGCAGCGA GAGGACAAGG CTGGGACGG GAATGGCCTA TGGACAGGCG	scrFI mvaI ecorII dsav bstNI apyl(dcm+) bsavI aluI mnlI hhaI/cfoI hinPI bssSI acII mspI bsII
4901	CTTCTCCCT TCGGGAAGCG TGGCGGTTT TCATAGCTCA CGCTGTAGT ATTCAGTTC GGTGTAGTTC GTTCGTCCA AGCTGGGCTG TGTGCAGAA GAAAGAGGA AGCCCTTCG ACCGCGAAG AGTATCGAGT GCGACATCCA TAGAGTCAAG CCACATCCAG CAAGCGAGGT TGACCCGAC ACAGGTGCTT	hinPI hhaI/cfoI haeII aluI scfI dclI alul apaLI/snoI alw4I/snoI
5001	CCCCCGTTC AGCCGACCG CTGGCGCTTA TCCGTAAT ATCGTCTTGA GTCCACCCG GTAAGACACG ACTTATCCGC ACTGGCAGCA GCCACTGCTA GGGGGGCAAG TCGGGCTGC GACCGCGAAT AGGCCATTGA TAGCAGAACT CAGGTGTCG CATTCGTGC TGAATAGCGG TGACCGTCGT CGGTGACCAT	fnu4HI bsolI nspBII acII hinPI mcrI bbvI bslEI maeIII mspl bsaWI pleI hinfi cauII nclI dsav bsolI bbvI bsrI bbvI bsrI maeIII
5101	ACAGGATTAG CAGAGCGAGG TATGTAGGCG GTGTCACAGA GTTCTTGAAG TGGTGGCTTA ACTACGGCTA CACTAGAGG ACGTATTTG GTATCTGCCG TGTCTAATC GTCTCGCTCC ATACATCCG CACGATGTCT CAAGAATTC ACCACCGAT TGAATCCGAT GTGATCTTCC TGTATAAAC CATAGACGCG	mnlI acII scfI bslI haeIII/palI haeI rmaI maeI bfaI hinPI hhaI/cfoI

FIG. 41Q

67 / 136

[illegible]

FIG. 41R

[illegible]

FIG. 41S

6001 ATTCTCTTAC TGTCATGCCA TCCGTAAGAT GCTTTTCTGT GACTGGTGAG TACTCAACCA AGTCATTCTG AGAATAGTGT ATGCGCGGAC CGAGTTGCTC
 TAAGAGAATG ACAGTACGGT AGGCATTCTA CGAAAGACA CTGACCACTC ATGAGTTGGT TCAGTAAGAC TCTTATCACA TACGCGGCTG GCTCAACGAG

6101 TTGCGCGGCG TCAACACGGG ATAATACCGG GCCACATAGC AGAAGTCTTAA AAGTGCTCAT CATTGGAAAA CGTTCTTCGG GCGGAAAACT CTCAGAGGATC
 AACGGGCGCG AGTTGTGCC TATTATGGCG CGGTGTATCG TCTTGAATTT TTCACGAGTA GTAACCTTTT GCAAGAAGCC CCGCTTTTGA GAGTTCCTAG

6201 TTACCGCTGT TGAGATCCAG TTCGATGTAA CCCACTCGTG CACCAACTG ATCTTCAGCA TCTTTTACTT TCACCAGCGT TTCTGGGTGA GCAAAAACAG
 AATGGCGACA ACTCTAGGTC AAGCTACATT GGTGAGCAC GTGGTTGAC TAGAAGTCGT AGAAATGAA AGTGGTCGCA AAGACCCACT CGTTTTTGTG

6301 GAAGGCAAAA TGCCGCMAAA AAGGGAATAA GGGCGACACG GAAATGTTGA ATACTCATAC TCTTCTCTTT TCAATATTAT TGAAGCATTT ATCAGGGTTA
 CTTCGGTTTT ACGGCGTTTT TTCCCTTATT CCCGCTGTGC CTTTACAAT TATGAGTAG AGAAGGAAA AGTATATAA ACTTCGTAA TAGTCCCAAT

69/136

FIG. 41T

71 / 136

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>length: 6563

aatII(GACGTC):      1645 6489
acc65I(GGTACC):      403 823
accI(GTHKAC):      1093 1963 4449
accIII(TCCGGA):      3867[dam-]
acII(CCGC):      178 542 805 877 1340 1750 1826 2011 2039 2043 2182 2242 2384 2492 2501 2504
                2628 2781 2784 2787 2906 2926 3005 3045 3094 3141 3226 3241 3309 3342 3367 3412
                3436 3448 3490 3544 3597 3613 3619 3700 3838 3967 3970 3981 4139 4155 4210 4266
                4351 4390 4400 4442 4467 4505 4518 4544 4561 4604 4611 4632 4723 4751 4878 4897
                5018 5128 5263 5272 5634 5725 5916 5962 6083 6127 6204 6313 6412 6459
acyI
see hlnI
afIIII(ACRYGT):      1307 4678
ageI(ACCGGT):      1788
ahaII/bsaHI(GRCGYC): 1645 1813 2616 2637 2751 3408 6107 6489
ahaII/draI(TTTAA):  5435 5454 6146
ahdK/eam1105I(GACNNNNNGTC): 346 5566
aluI(AGCT):      72 121 252 320 398 532 589 648 1126 1144 1167 1325 1386 1906 2054 2075 2126
                2218 2233 2889 3292 4202 4259 4270 4319 4338 4619 4845 4935 4981 5238 5759 5859
                5922
alw44I/snoI(GTGCAC): 1831 4494 4992 6238
alwI[dam-](GGATC):  412 413 712 713 1171 1471 2578 2579 3300 3870 5245 5319 5331 5416 5429 5893
                6196 6214
alwNI(dcm-)(CAGNNNCTG): 1117 1385 5089
apaI(GGGCCC):      1695
apaLI/snoI(GTGCAC): 1831 4494 4992 6238
apoI(RAATY):      1 391 4093
apyI(dcm+)(CCWGG): 640 999 1347 1357 1449 1665 1713 1755 1764 2333 3262 3645 4705 4826 4839
aseI/asnI/vspI(ATAAT): 5742
asnI
see aseI
asp700(GAANNNTTC): 905 930 4234 6166
asp718(GGTACC):      403 823
aspHI
see hgiAI
see tthlII
asui(GGNCC):      1119 1195 1425 1434 1446 1512 1695 1696 1752 2155 2375 2727 3002 3090 3139 3463

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FIG. 41V

72 / 136

Stop Template Primer

SL.97.2 5' CAT GGT ATA GGT TAA ACT TAT TTA CAC 3'

NNS Randomization Primer

SL.97.3 5' CAT GGT ATA GGT NNS ACT TAT TTA CAC 3'

FIG. 42

73 / 136

Randomization of Position N35 of Variable Light Chain CDR-1
Amino Acid Frequency

Phage Display (NNS Codon Library) Sort #3

Amino Acid	Frequency	% Total	IC50 (nM)
Asparagine (wt)	1	5.6	4.9
Glycine	6	16.6	3.1
Aspartic Acid	3	16.6	3.1
Glutamic Acid	4	22.2	0.1
Alanine	2	5.6	0.2
Lysine	1	5.6	ND
Serine	1	1.9	ND

FIG. 43A

74 / 136

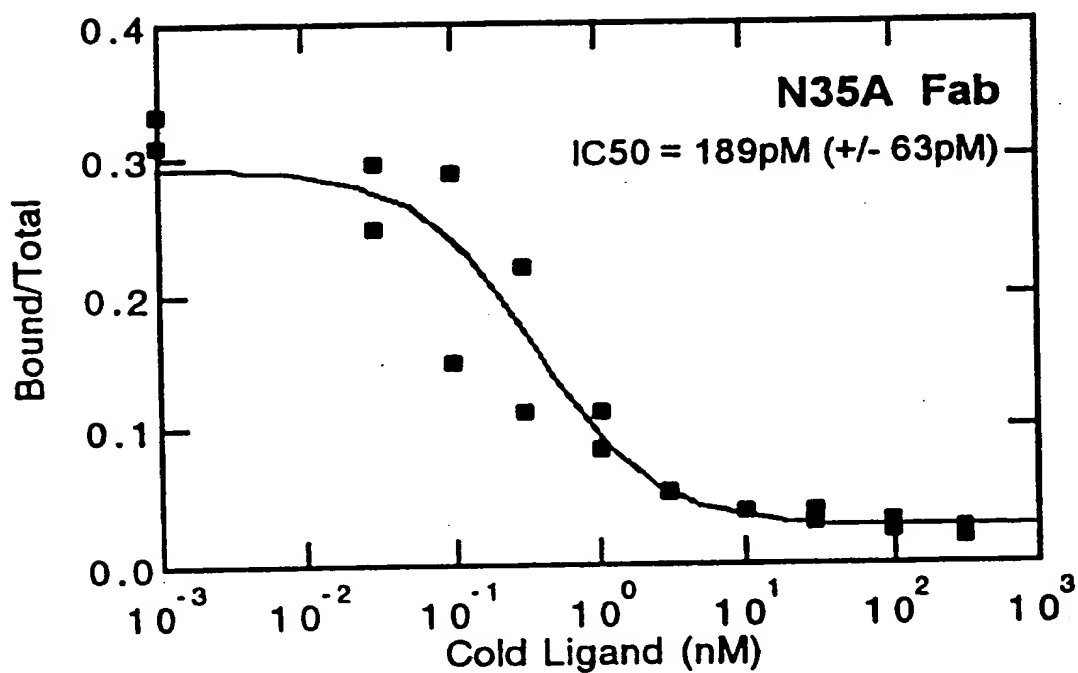


FIG. 43B-1

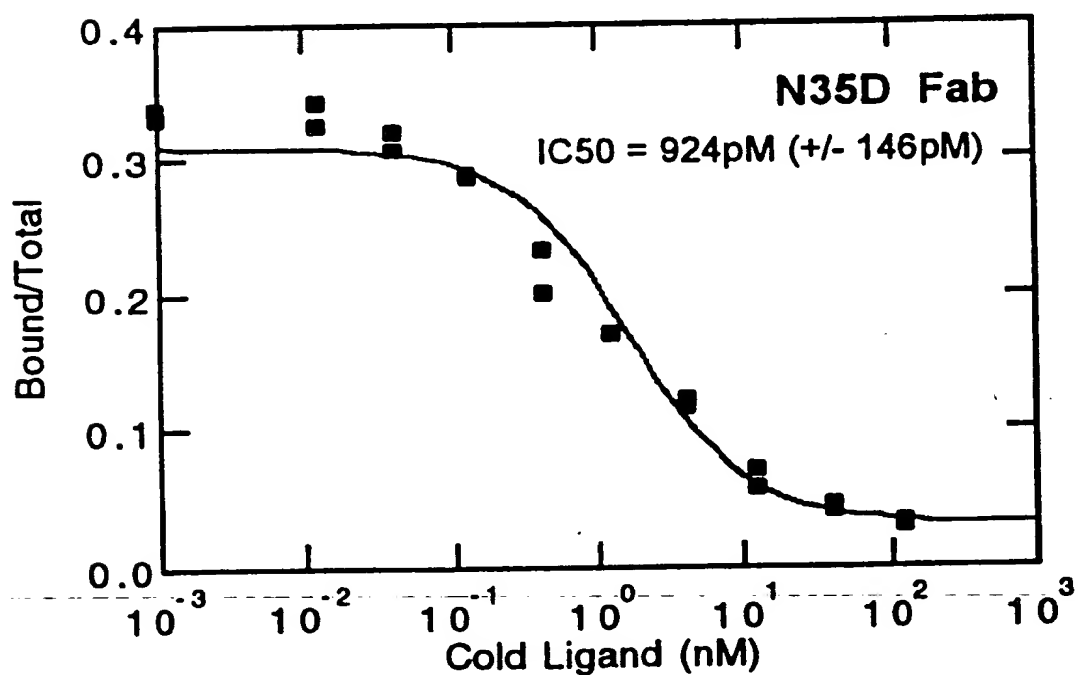


FIG. 43B-2

75 / 136

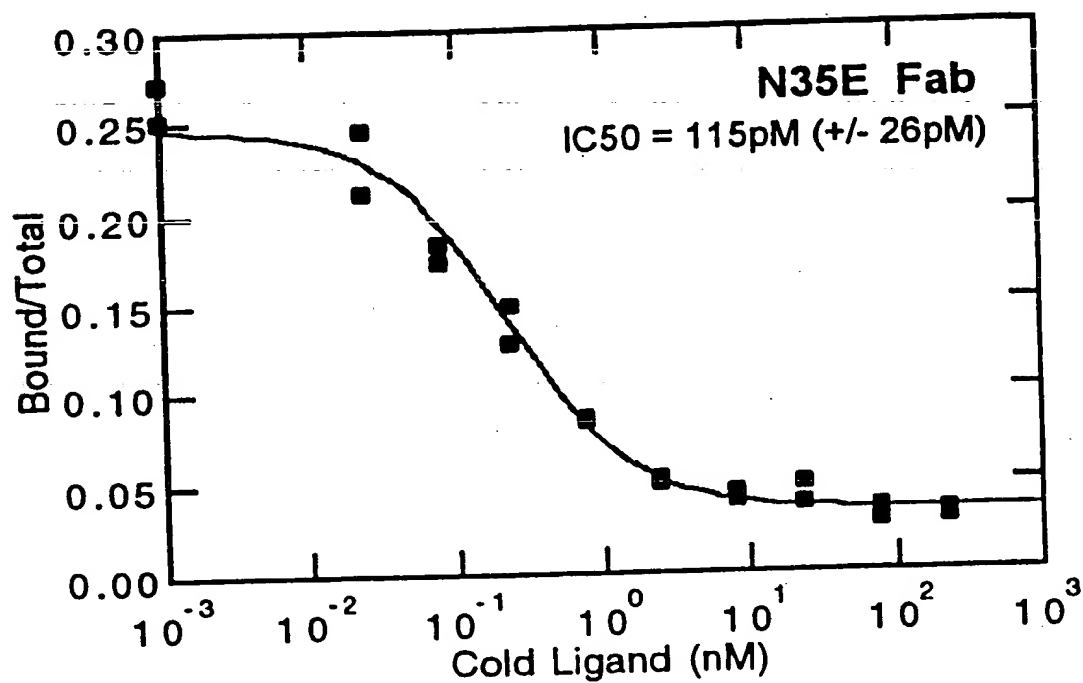


FIG. 43B-3

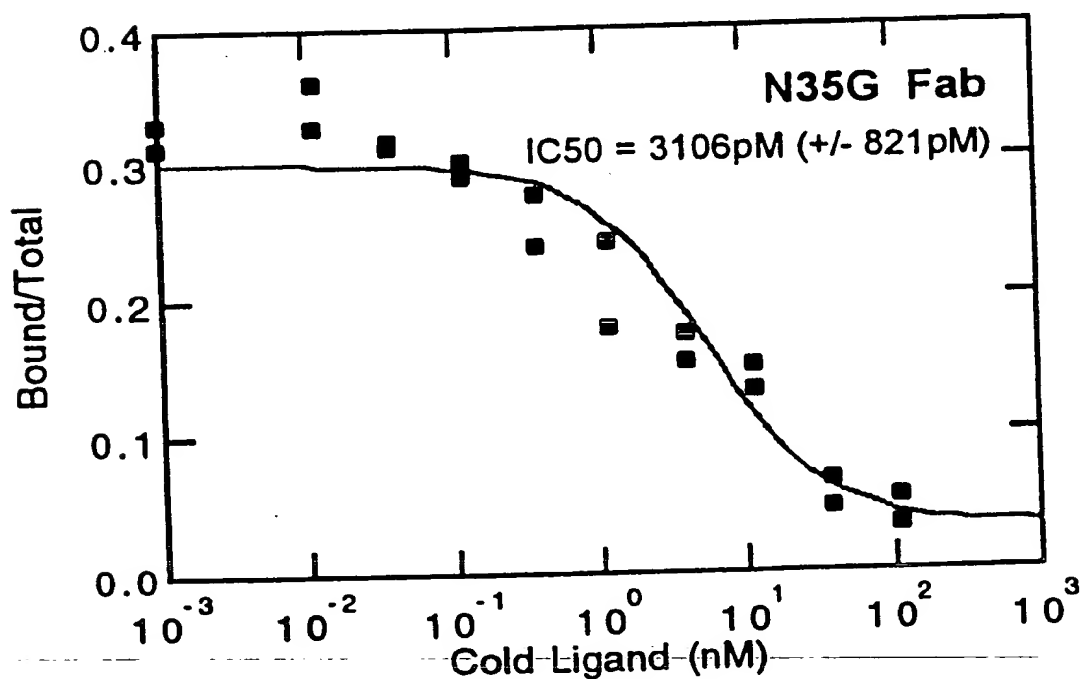
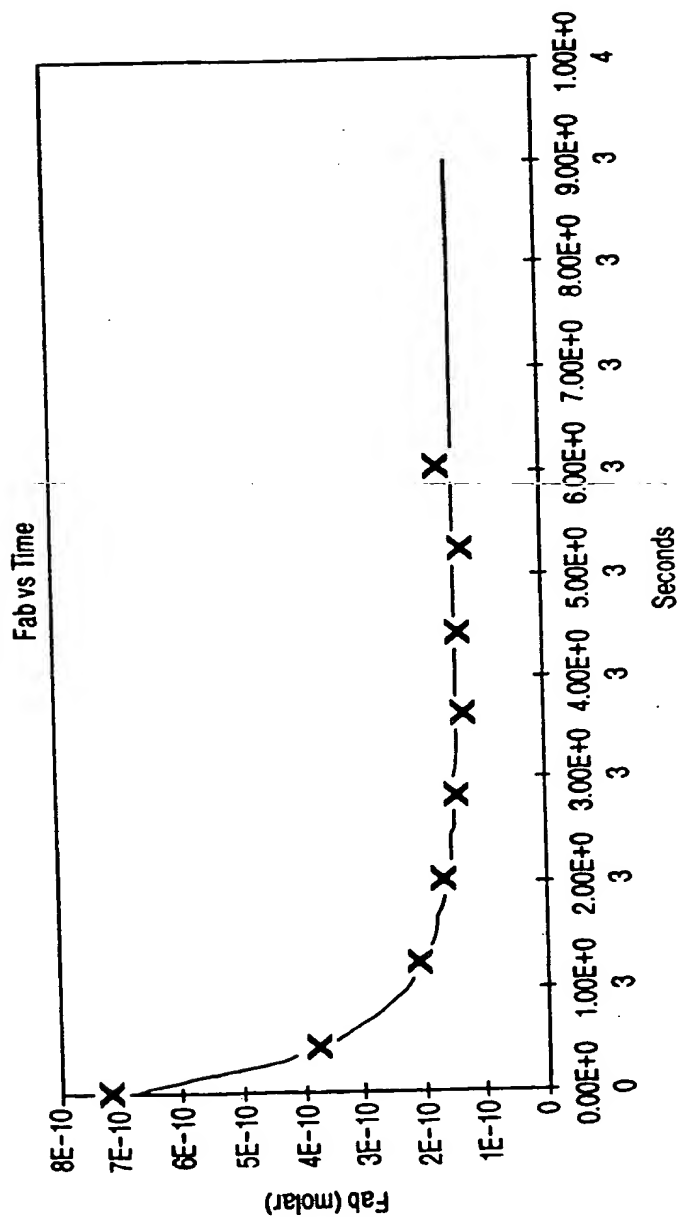


FIG. 43B-4

76/136



Representative Conc versus Time Plot. Shown is the kinetic data for 6G4V11N35A.F(ab')₂.

SAMPLE	ka	kd	Kd
6G4V11N35A-Fab	ND	ND	114pM
6G4V11N35A-F(ab') ₂	2.0x10 ⁶	2.1x10 ⁻⁴	109pM
6G4V11N35E-Fab	4.7x10 ⁶	2.6x10 ⁻⁴	54pM

FIG. 44

77 / 136

1 ATGAAAAAGA ATATCGCATT TCTTCTTGCA TCTATGTTTCG TTTTTCCTAT TGCTACAAAC
 TACTTTTTCT TATAGCGTAA AGAAGAACGT AGATACAAGC AAAAAAGATA ACGATGTTTG
 -23 M K K N I A F L L A S M F V F S I A T N
 61 GCATACGCTG ATATCCAGAT GACCCAGTCC CCGAGCTCCC TGTCCGCCTC TGTGGGCGAT
 CGTATGCGAC TATAGGTCTA CTGGGTCAGG GGCTCGAGGG ACAGGCGGAG ACACCCGCTA
 -3 A Y A D I Q M T Q S P S S L S A S V G D
 121 AGGGTCACCA TCACCTGCAG GTCAAGTCAA AGCTTAGTAC ATGGTATAGG TGAGACGTAT
 TCCCAGTGGT AGTGGACGTC CAGTTCAGTT TCGAATCATG TACCATATCC ACTCTGCATA
 18 R V T I T C R S S O S L V H G I G E T Y
 181 TTACACTGGT ATCAACAGAA ACCAGGAAAA GCTCCGAAAC TACTGATTTA CAAAGTATCC
 AATGTGACCA TAGTTGTCTT TGGTCCTTTT CGAGGCTTTG ATGACTAAAT GTTTCATAGG
 38 L H W Y Q Q K P G K A P K L L I Y K V S
 241 AATCGATTCT CTGGAGTCCC TTCTCGCTTC TCTGGATCCG GTTCTGGGAC GGATTTCACT
 TTAGCTAAGA GACCTCAGGG AAGAGCGAAG AGACCTAGGC CAAGACCCTG CCTAAAGTGA
 58 N R F S G V P S R F S G S G S G T D F T
 301 CTGACCATCA GCAGTCTGCA GCCAGAAGAC TTCGCAACTT ATTACTGTTC ACAGAGTACT
 GACTGGTAGT CGTCAGACGT CGGTCTTCTG AAGCGTTGAA TAATGACAAG TGTCTCATGA
 78 L T I S S L Q P E D F A T Y Y C S O S T
 361 CATGTCCCGC TCACGTTTGG ACAGGGTACC AAGGTGGAGA TCAAACGAAC TGTGGCTGCA
 GTACAGGGCG AGTGCAAACC TGTCCCATGG TTCCACCTCT AGTTTGCTTG ACACCGACGT
 98 H V P L T F G Q G T K V E I K R T V A A
 421 CCATCTGTCT TCATCTTCCC GCCATCTGAT GAGCAGTTGA AATCTGGAAC TGCTTCTGTT
 GGTAGACAGA AGTAGAAGGG CCGTAGACTA CTCGTCAACT TTAGACCTTG ACGAAGACAA
 118 P S V F I F P P S D E Q L K S G T A S V
 481 GTGTGCCTGC TGAATAACTT CTATCCCAGA GAGGCCAAAG TACAGTGGAA GGTGGATAAC
 CACACGGACG ACTTATTGAA GATAGGGTCT CTCCGGTTTC ATGTCACCTT CCACCTATTG
 138 V C L L N N F Y P R E A K V Q W K V D N
 541 GCCCTCCAAT CGGGTAACTC CCAGGAGAGT GTCACAGAGC AGGACAGCAA GGACAGCACC
 CGGGAGGTTA GCCCATGAG GGTCTCTCA CAGTGTCTCG TCCTGTCGTT CCTGTCGTGG
 158 A L Q S G N S Q E S V T E Q D S K D S T
 601 TACAGCCTCA GCAGCACCTT GACGCTGAGC AAAGCAGACT ACGAGAAACA CAAAGTCTAC
 ATGTCGGAGT CGTCGTGGGA CTGCGACTCG TTTCGTCTGA TGCTCTTTGT GTTTCAGATG
 178 Y S L S S T L T L S K A D Y E K H K V Y
 661 GCCTGCGAAG TCACCCATCA GGGCCTGAGC TCGCCCGTCA CAAAGAGCTT CAACAGGGGA
 CGGACGCTTC AGTGGGTAGT CCCGGACTCG AGCGGGCAGT GTTTCTCGAA GTTGTCCCTT
 198 A C E V T H Q G L S S P V T K S F N R G
 721 GAGTGTTAAG CTGATCCTCT ACGCCGGACG CATCGTGGCC CTAGTACGCA ACTAGTCGTA
 CTCACAATTG GACTAGGAGA TGCGGCCTGC GTAGCACCAG GATCATGCGT TGATCAGCAT
 218 E C O

FIG. 45

78 / 136

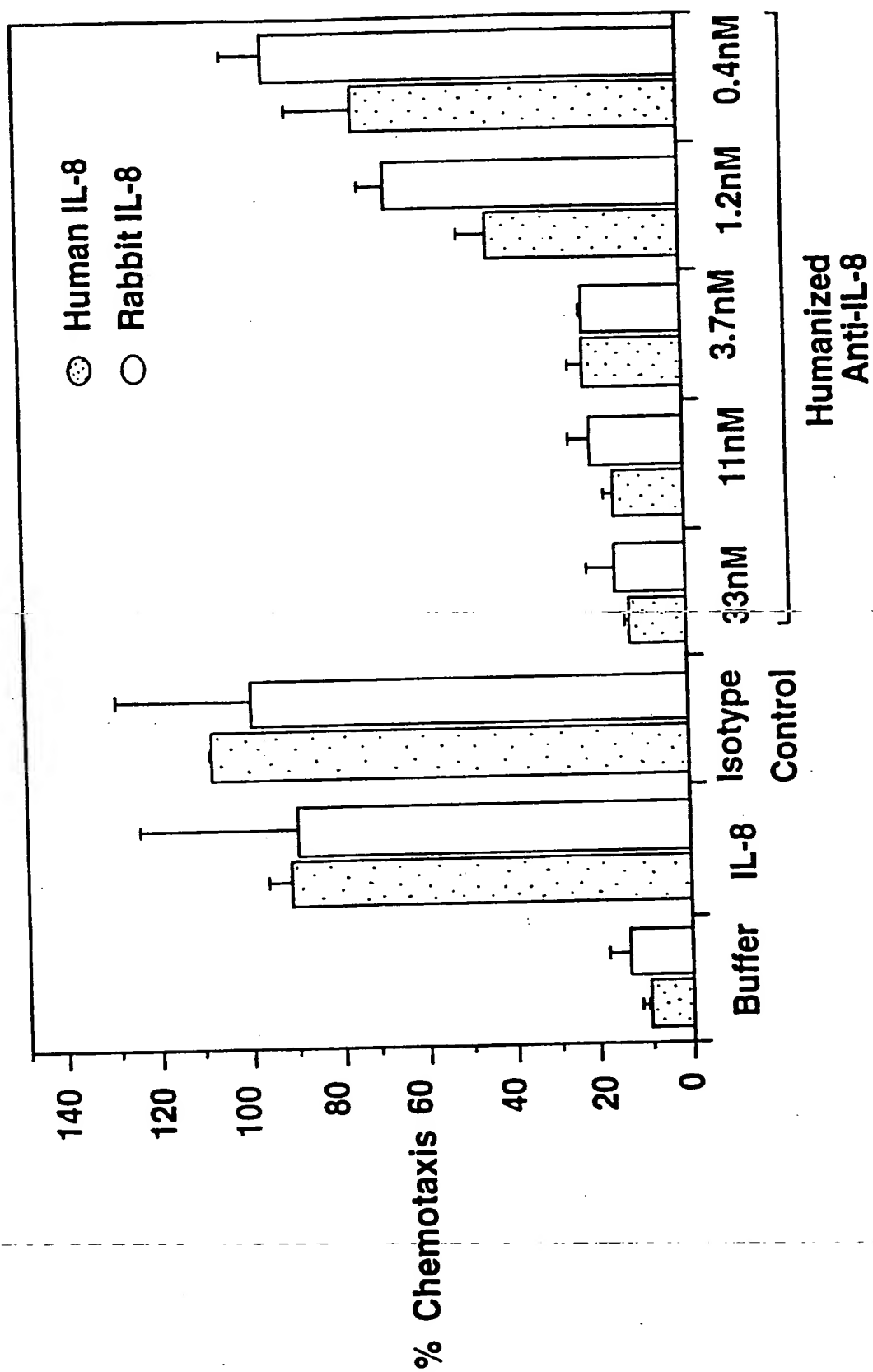


FIG. 46

79 / 136

N35AH1upr
5'-CTAGTGCAGTCTGGCGGTGGCCTGGTGCAGCCAGGGGGCTCACTCCGTTTGTCTGTGCAGCTTCTGGCTACTCCTTC-3'

N35AH1lwr
5'-TCGAGAAGGAGTAGCCAGAAGCTGCACAGGACAAACGGAGTGAGCCCCCTGGCTGCACCAGGCCACCGCCAGACTGCACT
AG-3'

Bold indicates nucleotide change destroying PvuII site.

FIG. 47

80 / 136

cac8I
 aluI
 sstI
 sacI
 hgiJII
 hgiAI/aspHI
 ecli36II
 bsp1286
 bsiHKAI
 bmyI
 banII
 taqI
 1 TTCGAGCTCG CCGGACATTG ATTATTGACT AGACTCGATC GACAGCTGTG GAATGTGTGT CAGTTAGGGT GTGGAAGATC CCCAGGCTCC CCAGCAGGCA
 AAGCTCGAGC GGGCTGTAC TAATAACTGA TCTCAGCTAG CTGTCGACAC CTTACACACA GTCAATCCCA CACCTTTTCAG GGTCCGAGG GGTGTCGTGT
 sau3AI aluI
 mboI/ndelII[dam-]
 dpnI[dam+]
 pvuI/bspCI
 pleI dpnII[dam-]
 hinfI taqI[dam-]
 rmaI mcrI pvuII
 maeI bsiEI nspBII
 bfaI taqI[dam-]
 bsmFI nlaIV
 cac8I
 sfaNI
 ppulOI
 nsiI/avaIII
 nlaIII
 sphI
 nspI
 nspHI
 cac8I
 bsmFI
 apyI[dcm+]
 bsaJI
 nspI
 nspHI
 cac8I
 nlaIII
 styI
 ncoI
 bsaI dsaI
 acII bsaJI
 acII bsaI acII
 acII foki
 acII
 bsmFI
 201 GTCAGCAACC ATAGTCCCGC CCCTAATCC GCCATCCCG CCCTAATC CCGCCAGTTC CGGCCATTCT CCGGCCCATG GCTGACTAAT TTTTITTTATT
 CAGTCTGTGG TATCAGGGCG GGGATTGAG CGGGTAGGCG GGGGATTGAG GCGGGTCAAG GCGGGGTAC GCGACTGATTA AAAAAAATAA

FIG. 48A

82 / 136

[illegible]

FIG. 48C

83 / 136

```

          hgaI
          hinII/acyI
          ahaII/bsaHI
scrFI      mnlI
mvaI      ecorII
dsav
bstNI     econI
          apyI[dcmt+] mnlI
          bsaJI      bslI ddeI
          901 GTGACAAGGA TCATGCAGGA ATTTGAAAGT GACACGTTTT TCCAGAAAT TGATTTGGG AAATATAAAC CTCTCCAGG ATACCAGGC GTCCTCTCTG
          CACTGTTCTT AGTAGCTCCT TAACTTTCA CTGTGCAAAA AGGCTCTTTA ACTAAACCCC TTTATATTG GAGAGGTCT TATGGGTCCG CAGGAGAGC
          maeII alwI[dam-] apoI      maeII      mnlI
          maeII      afIIII
          maeII      maeII
          1001 AGGTCACAGGA GGAAAAGGC ATCAAGTATA AGTTTGAAGT CTACGAGAAG AAGACTAAC AGGAAGATGC TTTCAGATTG TCTGCTCCC TCCTAAAGCT
          TCCAGGTCTT CCTTTTCCG TAGTTCATAT TCAAACTTCA GATGCTCTTC TTTCGATTG TCCTCTTACG AAAGTTCAAG AGACGAGGG AGGATTTCGA
          ^END DHER
          sfaNI      mboII      mnlI      aluI
          asuI      mnlI      accI      mboII
          1101 ATGCAATTTT ATAAGACCAT GGGACTTTTG CTGGCTTTAG ATCCCTTTGG CTTCGTTAGA AGCAGCTAC AATTATACA TAACCTTATG TATCATACAC
          TAGTAAAAA TATTCGTGGA CCTGAAAC GACCGAATC TAGGGAACC GAAGCAATCT TGGCTCGATG TTAATTATGT ATTGGAATAC ATAGTATGTG
          ppulOI      nsII/avaIII      cac8I      bstYI/xhoII
          nsII/avaIII      bsaJI      dsal bsmFI
          1101 ATGCAATTTT ATAAGACCAT GGGACTTTTG CTGGCTTTAG ATCCCTTTGG CTTCGTTAGA AGCAGCTAC AATTATACA TAACCTTATG TATCATACAC
          TAGTAAAAA TATTCGTGGA CCTGAAAC GACCGAATC TAGGGAACC GAAGCAATCT TGGCTCGATG TTAATTATGT ATTGGAATAC ATAGTATGTG
          sau96I      sau96I
          avall      avall
          asuI      asuI
          scrFI      scrFI
          mvaI      mvaI
          ecorII     ecorII

```

FIG. 48D

84 / 136

[illegible][illegible]

FIG. 48E

85 / 136

belI
 sau3AI
 mboI/ndeII[dam-]
 dpmI[dam+]
 dpmII[dam-]
 alwI[dam-] hphI
 1501 TGGGTTGGAT ATATTGATCC TTCCAATGGT GAACATGCGT ATAATCAAAA GTTCAAGGGC CGTTTCACIT TATCTCGCGA CAACCTCCAAA AHCACAGCAT
 ACCCAACCTA TATAACTAGG AAGGTACCA CTTTGATGCA CTTTGATGCA TATTAGTTTT CAAGTTCCCG GCAAAGTGAA ATAGAGCGCT GTTGAGGTTT TTCTGTCGTA
 47 W V G Y I D P S N G E T T Y N Q K F K G R F T L S R D N S K N T A Y
 scfI
 patI
 bagI
 cac8I mnlI
 cac8I ddeI drdI
 bspWI
 1601 ACCTGCAGAT GAACAGCCTG CGTGTGAGG ACACCTGCGT CTATTACTGT GCAAGAGGGG ATTATCGCTA CAATGTGTGAC TGGTCTTCGG AGCTCTGGGG
 TGGAGGTCTA CTTGTGGGAC GCACGACTCC TGTGACGGCA GATAATGACA CGTTCTCCCG TAATAGCGAT GTTACCACTG ACCAAGAGCG TGCAGACCCC
 81 L Q M N S L R A E D T A V Y Y C A R G D Y R Y N G D W F F D V W G
 sau96I
 nlaIV
 hgIJI
 bsp1286
 bsp120I
 bmyI
 banII
 asuI
 mboII ecorII
 apaI
 styI haeIII/pali
 bsaJI
 mnlI
 bsmBI
 1701 TCAAGGAACC CTGGTCACCG TCTCTCGGC CTCACCAAG GGCCCATCGG TCTTCCCTT GGCACCTCC TCCAAGAGCA CCTCTGGGG CACAGCGGCC
 AGTTCCCTGG GACCACTGGC AGAGAGCCG GAGGTGTTT CCGGTAGCC AGAAGGGGA CCGTGGAGG AGGTCTCGT GGAGACCCCC GTGTGCGCGS
 114 Q G T L V T V S S A S T K G P S V F P L A P S S K S T S G G T A A
 thai
 fnuDII/mvni
 bstUI
 bsh1236I
 nruI
 haeIII/pali
 sau96I
 asuI
 1801 TGGGTTGGAT ATATTGATCC TTCCAATGGT GAACATGCGT ATAATCAAAA GTTCAAGGGC CGTTTCACIT TATCTCGCGA CAACCTCCAAA AHCACAGCAT
 ACCCAACCTA TATAACTAGG AAGGTACCA CTTTGATGCA CTTTGATGCA TATTAGTTTT CAAGTTCCCG GCAAAGTGAA ATAGAGCGCT GTTGAGGTTT TTCTGTCGTA
 47 W V G Y I D P S N G E T T Y N Q K F K G R F T L S R D N S K N T A Y
 hinII/acyI
 ahaII/bsaHI
 aatII
 taqI
 berI
 maeIII
 hphI
 mboII maeII
 1901 TGGGTTGGAT ATATTGATCC TTCCAATGGT GAACATGCGT ATAATCAAAA GTTCAAGGGC CGTTTCACIT TATCTCGCGA CAACCTCCAAA AHCACAGCAT
 ACCCAACCTA TATAACTAGG AAGGTACCA CTTTGATGCA CTTTGATGCA TATTAGTTTT CAAGTTCCCG GCAAAGTGAA ATAGAGCGCT GTTGAGGTTT TTCTGTCGTA
 47 W V G Y I D P S N G E T T Y N Q K F K G R F T L S R D N S K N T A Y
 scrFI
 mvaI
 ecorII
 dsav
 bstNI
 bsaJI
 sau96I
 haeIII/pali
 asuI
 fnu4HI
 bsoFI
 bsp1286 acII bsaJI
 bmyI nspBII apyI[dcmt]
 1701 TCAAGGAACC CTGGTCACCG TCTCTCGGC CTCACCAAG GGCCCATCGG TCTTCCCTT GGCACCTCC TCCAAGAGCA CCTCTGGGG CACAGCGGCC
 AGTTCCCTGG GACCACTGGC AGAGAGCCG GAGGTGTTT CCGGTAGCC AGAAGGGGA CCGTGGAGG AGGTCTCGT GGAGACCCCC GTGTGCGCGS
 114 Q G T L V T V S S A S T K G P S V F P L A P S S K S T S G G T A A

FIG. 48F

86 / 136

1801 CTGGGCTGCC TGGTCAAGGA CTACTTCCC GAACCGGTGA CGGTGCTGTG GAACCTCAGC GCCTGACCA CGCGGTGCA CACTTCCG GCTGCTCCTAC
147 L G C L V K D Y F P E P V T V S W N S G A L T S G V H T F P A V L Q

1901 AGTCTCAGG ACTCTACTCC CTCAGCAGCG TGGTCACTGT GCCCTTAGC AGCTTGGCA CCCAGACCTA CATCTGCAAC GTGAATCACA AGCCAGCACA
181 S S G L Y S L S S V V T V P S S S L G T Q T Y I C N V N H K P S N

2001 GACCAAGGTG GACAAGAAAG TTGAGCCCAA ATCTTGTGAC AAACTCACA CATGCCACC GTGCCAGCA CCTGAACCTC TGGGGGACC GTCAGTCTTC
214 T K V D K K V E P K S C D K T H T C P P C P A P E L L G G P S V F

FIG. 48G

87 / 136

[illegible]

FIG. 48H

88 / 136

[illegible]

FIG. 48I

3301

```
AGGAGGCTTT TTTGAGGCC TAGGCTTTG CAAAAGCTA GCTTATCCGG CCGGACCGG TGCATTGGAA CGCGGATTCC CCGTGCAAG AGTCAGGTAA
TCCCTCCGAA AACCTCCGG ATCCGAAAC GTTTTTCGAT CGAATAGGCC GCGCCCTGCC ACGTACCTT CCGCTAAGG GGCACGTTT TCAGTCCATT
^seq from pSV16B5-6G4VL: AvrII - HindIII frag
^removed splice donor^

rmal mael styI bsaJI bnl avrII[dam-] haelII/pali stuI hael mnlI bfaI
alul rmal mael bfaI nheI cac8I alul
alul mcrI esgI/xmaIII/ecI XI eaeI cfrI bsiEI mspI caulI hpaII
haeIII/pali haeIII/pali mcrI esgI/xmaIII/ecI XI eaeI cfrI bsiEI mspI caulI hpaII
tflI hinfI acII thal fnuDII/mvnI bstUI bsh1236I pleI hinfI
dsav
```

3401

```
ATCTCAGATA TCCGGGTGG GGAACCGAAG CAATCTTCGG CCGATGTTAA TTATGTATTG GAAACCTAG CTAGGATGAC TGTGACTGTA
GTACCGGCTA TAGAGTCTAT AGGCCACCC CCTTGGCTTC GTTAGACGC GGTACAAT ANTACATAAC CTTTGGATC GATCCTACTG ACATGACAT
^U2 match
^sp6 promoter
^removed ATG
^U2 match
larlat consensus^
IgG vH natural larlat restored^
```

FIG. 48L

92 / 136

[illegible]

scfI
pstI
bsgI
sse8387I
hphI
maeIII
bsteII
hphI bspMI
alul csp6I
hindIII
ddeeI
rsal
maeII
snaBI
bsaAI
bsrI
bsstNI aluI
apyI(dcm+)
ecorII
dsav
mval
scfII

3701 ATAGGCTCAC CATCACTGC AGGTCAGTC AAAGCTTAGT ACATGGTATA GGTCCTACGT ATTTACACTG GTATCAACAG AAACAGGAA AAGCTCCGAA
TATCCCACTG GTAGTGACG TCCAGTTCAG TTTCGGAATCA TGTACCATAT CCACGATGCA TAAATGTGAC CATAGTTGTC TTTGTGCTTT TTCGAGGCTT

18 R V T I T C R S S Q S L V H G I G A T Y L H W Y Q Q K P G K A P K

FIG. 48M

93 / 136

```

mspl
hpaII
bali
bsaWI
sau3AI
mboI/ndeII[dam-]
dpmI[dam+]
dpmII[dam-]
alwI[dam-]
nlaIV
bstYI/xhoII
bamHI
alwI[dam-]
bamFI
3801 ACTACTGATT TACAAAGTAT CCAATCGATT CTCTGGAGTC CCTTCTCGCT TCCTCTGGATC CGGTCTGGTGG ACGGATTCA CTCTGACCAT CAGCAGTCTG
TGATGACTAA ATGTTTCATA GGTAGCTAA GAGACCTCAG GGAAGAGGGA AGACACCTAG GCCAAGACCC TGCCTAAGT GAGACTGGTA GTCGTCAGAC
51 L L I Y K V S N R F S G V P S R F S G S G S G T D F T L T I S S L
tfII
hinfi
taqI
clai/bsp106 pleI
bspDI[dam-] hinfi
3901 CAGCCAGAAG ACTTCGCAAC TTATTACTGT TCACAGAGTA CTCATGTCCC GCTCAGCTTT GGACAGGTA CCAAGGTGGA GATCAACGA ACTGTGGCTG
GTGCTCTTC TGAAGGTTG AATAAGACA AGTGTCTCAT GAGTACAGGG CGAGTGCATA CCTGTCCCAT GGTCCACCT CTAGTTTGT TGAACCCGAC
84 Q P E D F A T Y Y C S Q S T H V P L T F G Q G T K V E I K R T V A A
mboII
bpuAI
bbsI
4001 CACCATCTGT CTTCATCTTC CGCCATCTG ATGACCAGTT GAAATCTGGA ACTGCTTCTG TTGTGTGCTT GCTGAATAAC TTCTATCCCA GAGAGGCCAA
GTGGTAGACA GAAGTAGAAG GGGGTAGAC TACTGCTCAA CTTTAGACCT TGACGAACGA AACACACGGA CGACTATTG AGATAGGCT CTCTCCGTT
118 P S V F I F P P S D E Q L K S G T A S V V C L L N N F Y P R E A K

```

FIG. 48N

94 / 136

```

151 V Q W K V D N A L Q S G N S Q E S V T E Q D S K D S T Y S L S S T
      real      mnlI      maeIII      maeIII      scfI mnlI bsvI
      csp6I      belI      GTGTACACAGA GTGTACACAGA GCAGGACAGC AAGCACAGCA CCTACAGCCT CAGCAGCACC
4101 AGTACAGTGG AAGGTGGATA AGCCCTCCCA ATCGGGTAAC TCCAGGAGA GTGTACACAGA GCAGGACAGC AAGCACAGCA CCTACAGCCT CAGCAGCACC
      TCATGTCCACC TTCCACCTAT TCGGGGAGGT TAGCCCATTG AGGTCTCTCT CACAGTGTCT CACAGTGTCT CACAGTGTCT CACAGTGTCT CACAGTGTCT
184 L T L S K A D Y E K H K V Y A C E V T H Q G L S S P V T K S F N R G
      ddeI      celII/espI      hgeI      hphI      maeIII      maeIII      maeIII      aluI
      bplI/bpull102I      accI      cac8I      maeIII      alwNI[dcn-]      maeIII      aluI
      haeIII/pali      sau96I aluI      asuI      banII      eco0109I/draII
4201 CTGAGGCTGA GCAAGCAGA CTACGAGAAA CACAAAGTCT AGGCTGGCA AGTCACCCAT CAGGGCCTGA GTCGCCCGT CACAAGAGC TTCAACAGGG
      GACTGCGACT CGTTTGTCT GATGCTCTTT GTGTTTACA TCGGACGCT TCAGTGGTA GTCCCGGACT CGAGGGGCA GTGTTCTCG AAGTTGCCC
184 L T L S K A D Y E K H K V Y A C E V T H Q G L S S P V T K S F N R G
      sau96I      acII      haeIII/pali      fnu4HI      asuI      bsofI      nlaIII
      sfiI      styI      aluI      haeIII/pali      hindIII      bglI      ncoI
      tru9I      eaeI      dsaI      maeIII      cfrI      bsaJI      maeIII      sfaNI      apoI
4301 GAGAGTGTTA AGCTTGCCG CCATGGCCCA ACTTGTTTAT TGCAGCTTAT AATGGTTACA AATAAGCAA TAGCATCACA AATTTCACA AATAAGCATT
      CTCTCACAAT TCGAACCGG GGTACCGGT TGAACAATA ACGTGGAATA TTACCAATGT TTATTCGTT ATCGTAGTGT TTAAGTGT TATTGCTAA
218 E C O

```

FIG. 480

4401 TTTTCACTG CATTCTAGTT GTGGTTTGTC CAAACTCATC AATGATCTT ATCATGCTG GATCGATCGG GAATTAATTC GCGCAGCAC CATGGCCTGA
AAAAAGTGAC GTAAGATCAA CACCAACACAG GTTGAGTAG TTACATAGAA TAGTAGAC CTAGCTAGCC CTTAATTAAG CCGCGTGTG GTACCGGACT
~sv40 origin

4501 AATAACCTCT GAAAGAGGAA CTTGGTTAGG TACCTTCTGA GCGGAAAGA ACCAGCTGTG GAATGTGTGT CAGTTAGGTG GTGGAAGTC CCCAGGCTCC
TTATTGGAGA CTTTCTCCTT GAACCAATCC ATGGAAGACT CGCGCTTTCT TGTCGACAC CTTACACACA GTCAATCCCA CACCTTTCAG GGTCCGAGG

4601 CCAGCAGGCA GAAGATGCA AAGCATGCAT CTCAATTAGT CAGCAACCAG GTGTGGAAG TCCCCAGCT CCCAGCAGG CAGAGTATG CAAAGCATGC
GGTCGTCCGT CTTCTACGT TTCGTACGTA GACTTAATCA GTCGTTGGTC CACACCTTC CACAGGTCGA GGGGTCCGA GGGGTCCGTC GTCTTCATAC GTTTCGTACG

FIG. 48P

[illegible]

FIG. 48Q

97 / 136

sau3AI
 mboI/ndeII(dam-)
 dpnI(dam+)
 sau96I
 haeIII/palI
 asuI
 mnlI acII
 pvuI/bspCI
 mboII cac8I
 mcrI
 earI/ksp632I
 bslEI
 CGAAGAGGCC CGACCGGATC GGCCTTCCCA ACAGTTGGCT
 CGGCATTATC GCGTGGCTAG CCGGAAGGGT TGTCAACGCA
 GCTTCTCCGG
 cac8I
 aluI
 pvuII
 nspBII
 cac8I
 foki
 fnu4HI
 bsoFI
 bbvI
 tru9I
 msel
 maeIII
 CAACTTAATC GCCTTGACG ACATCCCCC TTGCGCAGCT GCGTAATAG
 TGTAGGGGG AGCGGTGCG
 CGAACGTCG
 hinPI
 hhaI/cfoI
 nlaIV
 narI
 kasi
 hinII/acyI
 hgiCI
 haeII
 acII
 bglI
 AGCCTGAATG GCGAATGGCG CCGTATGGCG TATTTCTCC TTACGCATCT GTGCGGTATT TCACACCGCA TACGTCAAAG CAACCATAGT ACGGCGCCTG
 CGCTTACCGC GGAATACGCG ATAAAGAGG AATGCGTAGA CACGCCATAA AGTGTGGCGT ATGCAGTTTC GTTGGTATCA TGGCGGGGAC
 fnu4HI
 bsoFI
 thal
 fnuDII/mvni
 bstUI
 bsh1236I
 maeIII bbvI maeIII
 cac8I
 bfaI
 cac8I
 mboII
 CTTTGGCITT CTTCCCTTCC
 TTAGCGGGCA TTAGCGCGG CGGTGTGTGT GGTACGCGC AGCGTGACCG CTACACTGC CAGCGCCCTA GCGCCCGTC CTTTGGCITT CTTCCCTTCC
 ATCGCCGCGT AATTGCGCGC GCCACACCA CCAATGCGG TCGCACTGC GATGTGACG GTGCGGGAT CCGGGCGAG GAAAGCGNA GAAGGNAGG

FIG. 48R

98 / 136

mspI nlaIV hglJII hpaII nlaIV hglCI taqI
 naeI bsp1286 bmyI banII mnlI
 cfr10I/barFI
 maeII cac8I aluI
 maeII haeIII/palI
 draIII sau96I
 bsaAI asuI
 hphI
 5301 TTCTCTGCCA CGTTGCGCGG CTTTCCCGT CAAGCTCTAA ATCGGGGCT CCCTTTAGG TTCCGATTGA GTGCTTTACG GCACCTCGAC CCCAAAAAAC
 AAAGAGCGGT GCAAGCGGCC GAAAGGGCA GTTCAGATT TAGCCCCCGA GGAATCCC AAGCTAAAT CACGAATGC CGTGAGCTG GGGTTTTTGTG
 maeII haeIII/palI
 draIII sau96I
 bsaAI asuI
 hphI
 5401 TTGATTGGG TGATGGTTCA CGTACTGGC CATCGCCCTG ATAGACGCTT TTTCGCCCTT TGAGTTGGA GTCCACGTC TTAATATAGT GACTCTTGT
 AACTAAACC ACTACCAAGT GCATCACCG GTAGCGGAC TATCTGCCA AAGCGGGAA ACTGCAACCT CAGGTGCAAG AATTATATC CTGAGAACA
 bsaI bslI auaI
 5501 CCAAACTGA ACAACACTCA ACCCTATCTC GGGCTATTCT TTGATTAT AAGGATTTT GCCGATTTTC GCCTATTGT TAAAAAATGA GCTGATTTAA
 GGTGTGACCT TGTTGTGAGT TGGGATAGAG CCCGATAAGA AACTAATA TTCCCTAAA CGGCTAAAGC CGGATACCA ATTTTACT CGACTAAAT
 hglAI/aspHI
 bsp1286
 bslHKAI
 bmyI ddeI
 apaLI/snoI rsaI
 alw44I/snoI csp6I
 5601 CAAAAATTA ACGCAATTT TAACAAATA TTAACGTTTA CAATTTTATG GTGCACTCTC AGTACAATCT GCTCTGATGC CGCATAGTTA AGCCAACTCC
 GTTTTAAAT TCGCGTTAA ATTGTTTAT AATTGCAAT GTTAAATATC CACGTGAGAG TCATGTAGA CGAGACTAG CGGTATCAAT TCGGTTGAGG
 hglAI/aspHI
 bsp1286
 bslHKAI
 bmyI ddeI
 apaLI/snoI rsaI
 alw44I/snoI csp6I
 5701 GCTATCGCTA CGTACTGGG TCATGGCTGC GCCCGACAC CCGCCACAC CGCTGACG GCCTGACGG GCCTGTCTGC TCCCGCATC CGCTTACAGA
 CGATAGCGAT GCATGACCC ACTACCGAG CGGGCTGTG GCGGCTGTG GCGCACTCG CGGCACTGCC CGACAGAGC AGGCGCTAG GGAATGTCT

FIG. 48S

[illegible]

FIG. 48U

66501 CGACGAGCGT GACACCACGA TGGCAGCAGC AATGGCAACG AGCTGGCGCA AACATTATAAC TGCCGAACTA CTTACTCTAG CTTCGCCGCA ACAATTATA
GCTGCTCGCA CTGTGTGCT ACGGTCGTCG TTACCGTTCT TGCAACGCCT TTGATAATTG ACCGCTTGAT GAATGAGATC GAAGGCCCGT TGTTAATTAT

6701 GACTGGATGG AGCGGATAA AGTTGCAGCA CCACCTCTGC GCTCGGCCCT TCCGGTGGC TGGTTTTATG CTGATAAATC TGGAGCCGGT GAGCGTGGGT
CTGACCTACC TCCGCCATT TCAACGTCCT GGTAAGACG CGAGCGCGGA AGCGCACCG ACCAATAAC GACTATTAG ACTCGGCCA CTCGCACCCA

6801 CTCGCGGTAT CATTCAGCA CTGGGGCCAG ATGGTAAGCC CTCCCCTATC GTAGTTATCT ACAGCAGCGG GAGTCAGGCA ACTATGGATG AAGCAAATAG
GAGCGGCATA GTAACGTCGT GACCCCGGTC TACCATTGG GAGGCGATAG CATCAATAGA TGTGCTGCC CTCAGTCCGT TGATACCTAC TTGCTTTATC

6901 ACAGATCGCT GAGATAGGTC CCTCACTGAT TAAGCATGG TAACGTGFCAG ACCAAGTTTA CTCATATATA CTTTAGATTG ATTAAACT TCATTTTAA
TGTCTAGCGA CTCATCCAC GGAGTGACTA ATTCGPAACC ATTGACAGTC TGGTTCAAT GAGTATATAT GAAATCTAAC TAAATTTTGA AGTAANAAT

7001 TTTAAAAGGA TCTAGTGNA GATCCCTTTT GATAATCTCA TGACCAAAAT CCCTTAACGT GAGTTTTCGT TCCACTGAGC GTGAGACCC GTGAGAAAGA
AAATTTTCT AGATCCACT CTAGGAAAA CTATTAGAT ACTGGTTTTA GGAATTCGA CTCRAAGCA AGGTGACTCG CAGTCTGGG CATCTTTCT

FIG. 48V

[illegible]

FIG. 48W

103/136

```

scrFI      mval      ecorII      dsav      bstNI      bsaJI      bsaSI      hinPI      hhaI/cfoI      aluI      apyI|dcm+|
7501 TGAGCATTGA GAACGGCCA CGCTTCCCGA AGGAGAAAG GCGACAGGT ATCCGGTAAG CGGCAGGGTC GGAACAGGAG AGCCACGAG GAGCTTCCA
ACTCGTAACT CTTCGGCGT GCGAAGGGCT TCCCTCTTTC CGCTGTCCA TAGGCATTC GCGGTCCCAG CTTGTCTC TCCTGCTC CCTCGAAGGT

scrFI      mval      ecorII      dsav      bstNI      bsaJI      bsaSI      hinPI      hhaI/cfoI      aluI      apyI|dcm+|
7601 GGGGAAACG CTTGTATCT TTATAGTCT GTCCGGTTTC GCCACTCTG ACTTGACGT CGATTTTGT GATGCTGTC AGGGGGCGG AGCCTATGGA
CCCCCTTGC GGACCATAGA AATATCAGGA CAGCCCAAAG CGGTGAGAC TGAATCGCA GGTAAACA CTACGAGCAG TCCCCCGCC TCGGATACCT

scrFI      mval      ecorII      dsav      bstNI      bsaJI      bsaSI      hinPI      hhaI/cfoI      aluI      apyI|dcm+|
7701 AAAAGCCAG CAACGGCGC TTTTACGGT TCCTGGCCTT TTGCTGGCT TTTGCTCACA TGTCTTTCC TCGGTTATCC CTTGATTCG TGGATAACCG
TTTGGGTC GTTGGCGG AAAATGCCA AGGACCGAA AACGCCGA AACGAGTGT ACAAGAAAG AGCAATAGG GGAATAAG ACCTATTGG

scrFI      mval      ecorII      dsav      bstNI      bsaJI      bsaSI      hinPI      hhaI/cfoI      aluI      apyI|dcm+|
7801 TATTACGCC TTTAGTGAG CTGATACCG TCGCCGAGC CGAACGACG GTCAGTGAG GAGGAGCGG AAGAGCGCC AATACGCAAA
AATATGGCG AACTCACTC GACTATGCG AGCGGCTG CTTGCTGC CAGTCACTC TCTCTCGG TTCTCGGTTT

```

FIG. 48X

104 / 136

```

thai
fnuDII/mvnI
bstUI
bsh1236I
hinPI
hhaI/cfoI
thai
fnuDII/mvnI          cac8I
bstUI    haeIII/paI  aluI
bsh1236I          tru9I  pvuII
mnlI    bslI    eaeI    tfII    aaeI/asnI/vsPI
aciI    cfrI    hinPI    maeI    nspBII
7901 CCGCCTCTCC CCGCGGTG GCGATTCTAT TAATCCAGCT GGCACGACAG GTTCCCGAC TGGAAAGCGG GCAGTGAGCG CAAGCAATT AATGTGAGTT
GGCGGAGAGG GCGCGCAAC CGGTAAGTA ATTAGGTGCA CCGTGCTGC CAAAGGGTG ACCTTTCGCC CGTCACCTGC GTTCGGTTAA TTACACTCAA
cac8I    hinPI    maeIII    tru9I
cac8I    aciI    hhaI/cfoI    aaeI/asnI/vsPI
berI
8001 ACCTCACTCA TTAGCACCC CAGGCTTAC ACTTATGCT TCCGGCTCGT ATGTTGTGTG GAATTGTGAG CGGATAACAA TTTCACACAG GAAACAGCTA
TGGAGTGAGT AATCCGTGGG GTCCGAAATG TGAATACGA AGGCCGAGCA TACAACAC CTTACACTC GCCTATTGTT AAAGTGTGTC CTTGTGCGAT
mspI    hpaII    aciI    bsrBI    aluI
nlaIV bstNI
hgiCI apyI(dcm+)
banI bsaJI
mnlI

```

FIG. 48Y

105 / 136

```

tru9I
mseI
aseI/asnI/vspI
xmnl
nlaIII asp700
8101 TGACCATGAT TACGAATTAA
ACTGCTACTA ATGCTTAATT

>length: 8120

aatII(GACGTC): 1690 5947
acc65I(GGTACC): 2969 3967 4529
823 1039 2738 4237
217 229 238 250 260 271 317 422 454 485 574 1385 1795 1871 2248 2250 2758 2982
3167 3179 3188 3200 3210 3221 3267 3372 3404 3449 3686 3949 4021 4318 4542 4727
4739 4748 4760 4770 4781 4827 4910 4914 5070 5127 5153 5166 5203 5217 5220 5248
5275 5680 5699 5741 5751 5790 5979 6026 6125 6234 6311 6355 6476 6522 6713 6804
7166 7175 7310 7420 7541 7560 7687 7715 7806 7827 7834 7877 7901 7911 7967 8070
see hlnII
acyI
afIII/bfII(CTAAG): 786
afIII(ACRYGT): 932 7758
ageI(ACCGGT): 1833
ahaII/baaHI(GRCGYC): 988 1690 1858 5117 5947 6329
ahaII/draI(TTTAA): 696 4935 6290 6982 7001
ehdI/eamI105I(GACNNNNNGTC): 2087 6865
5 44 332 386 390 753 1097 1165 1370 1431 1951 2603 2751 2784 3282 3336 3340
3562 3566 3676 3733 3792 4270 4288 4311 4344 4554 4842 4896 4954 5047 5333 5590
5803 5822 6516 6579 6679 7200 7457 7593 7819 7937 8096
aluI(AGCT):
alw44I/anoI(GTGCAC): 1876 5651 6198 7444

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FIG. 48Z

106 / 136

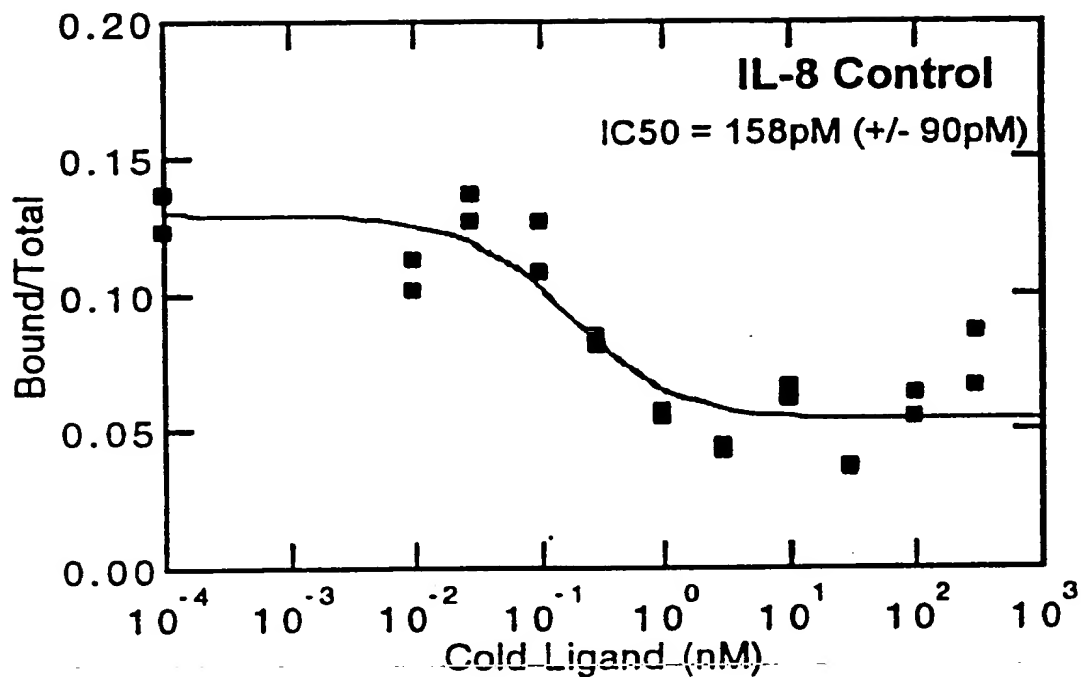


FIG. 49A

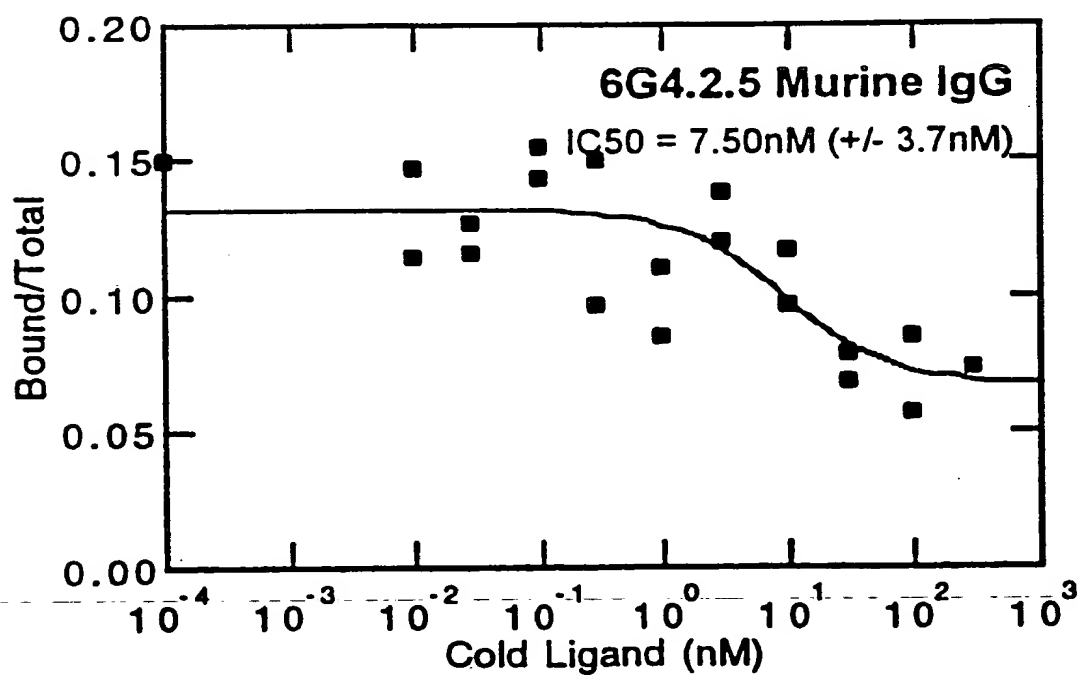


FIG. 49B

107 / 136

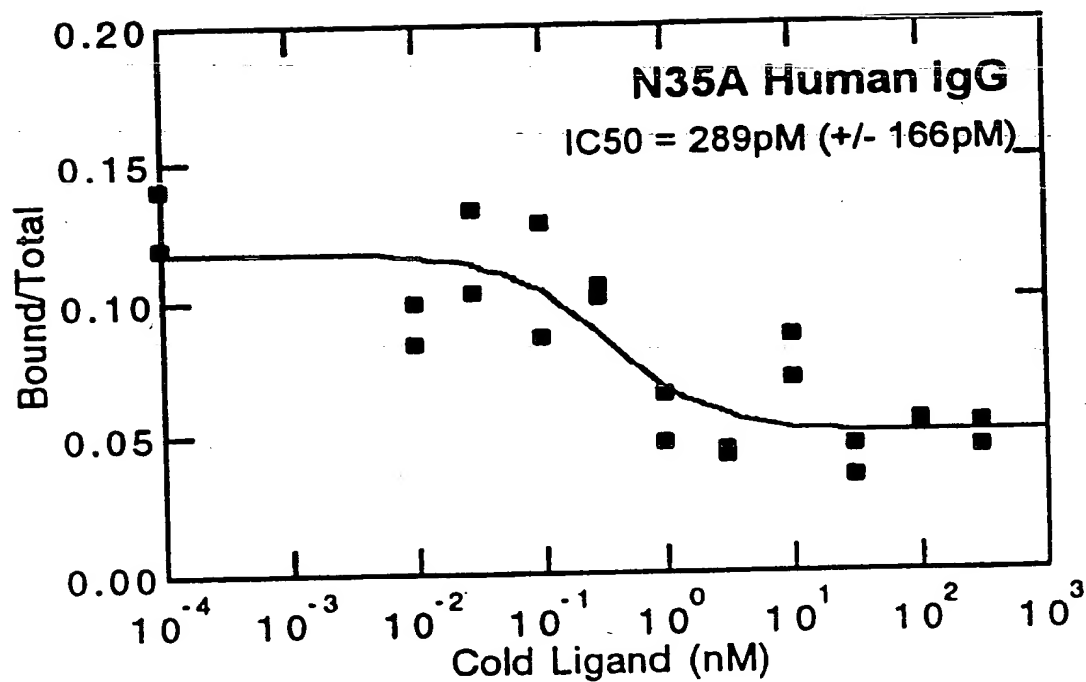


FIG. 49C

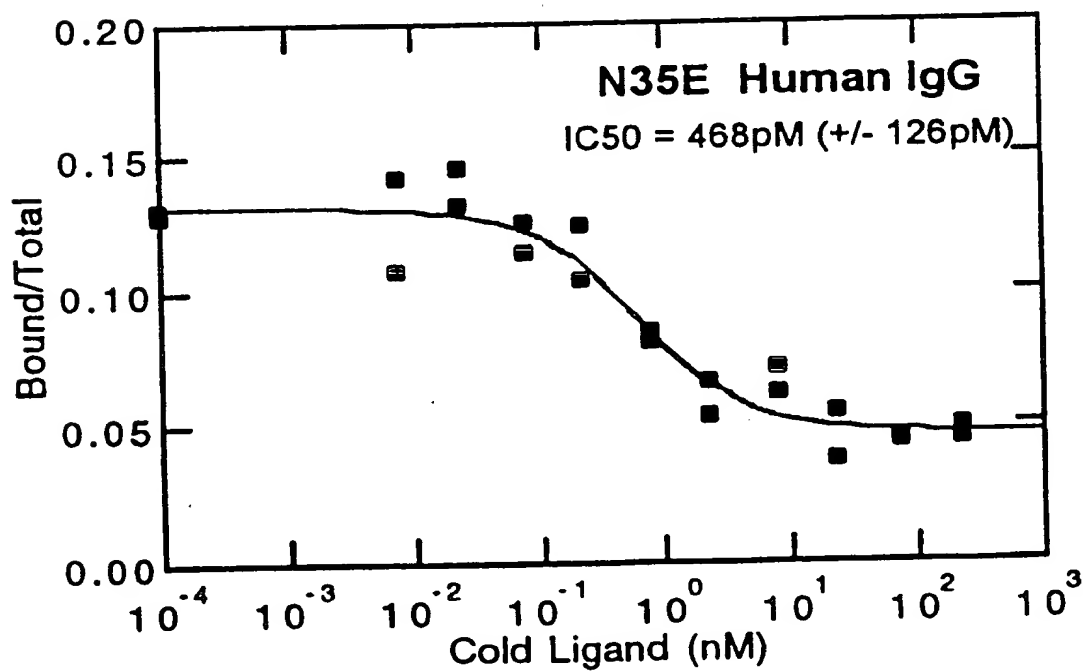


FIG. 49D

108 / 136

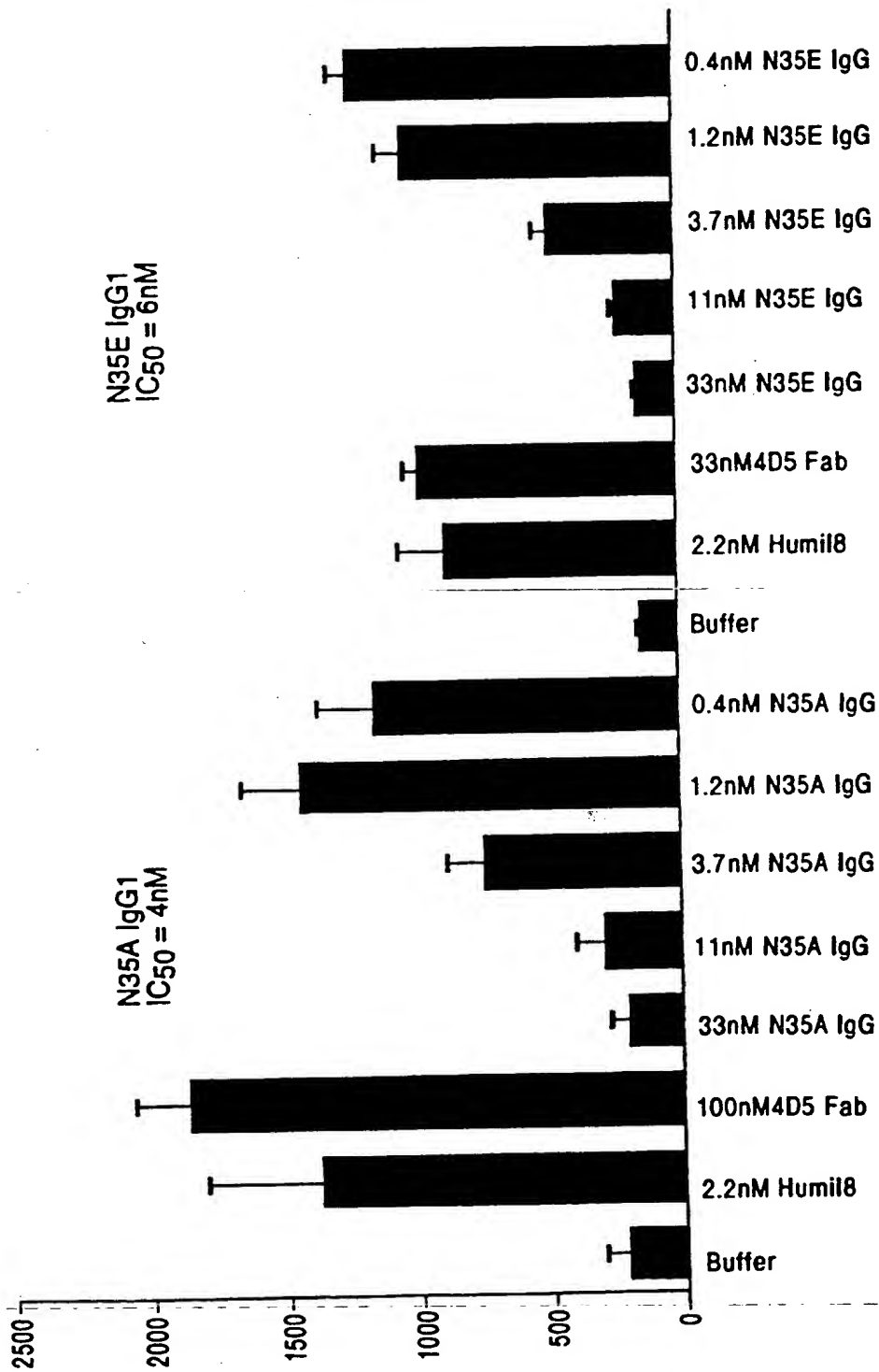


FIG. 50A

109 / 136

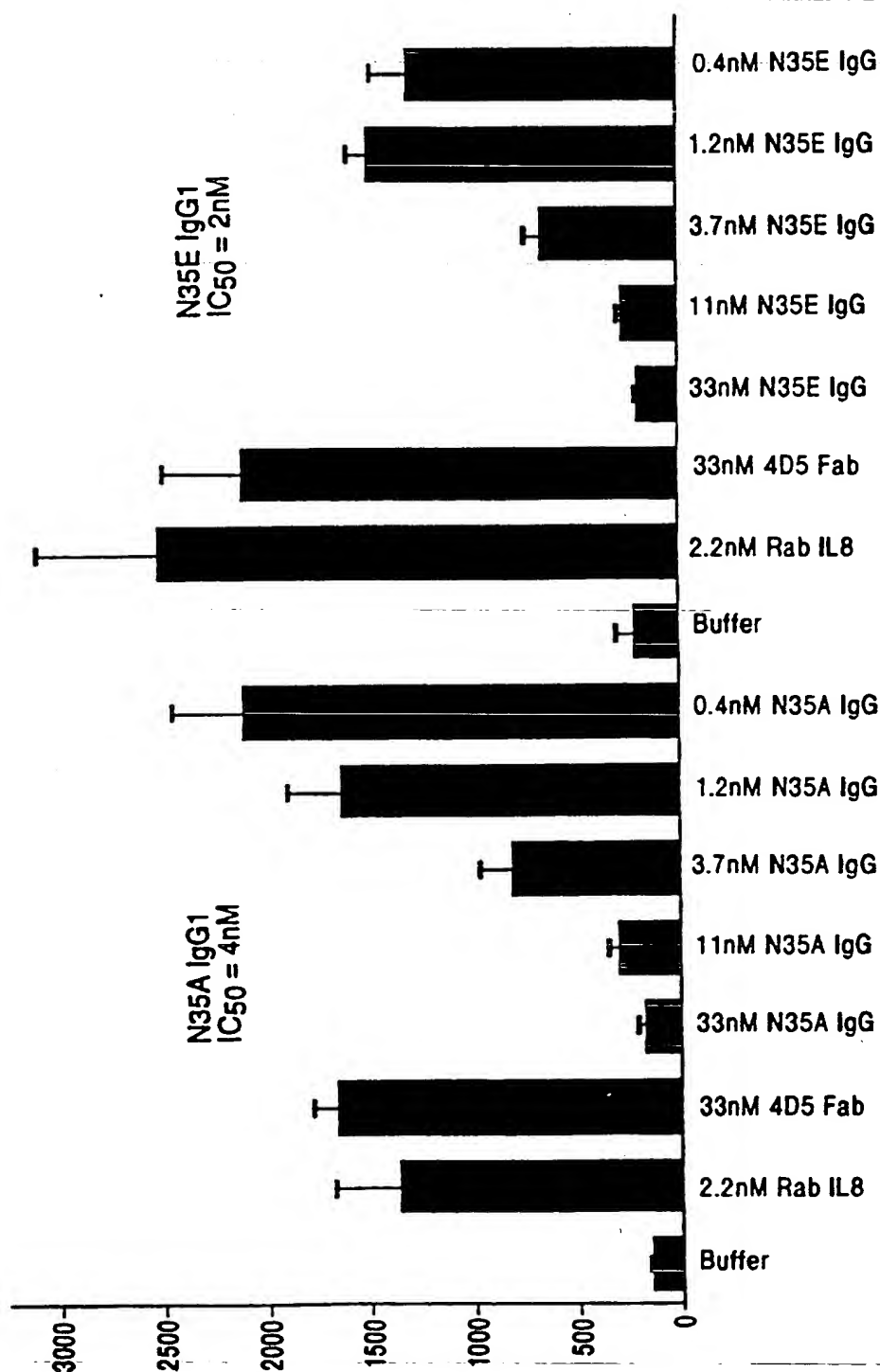
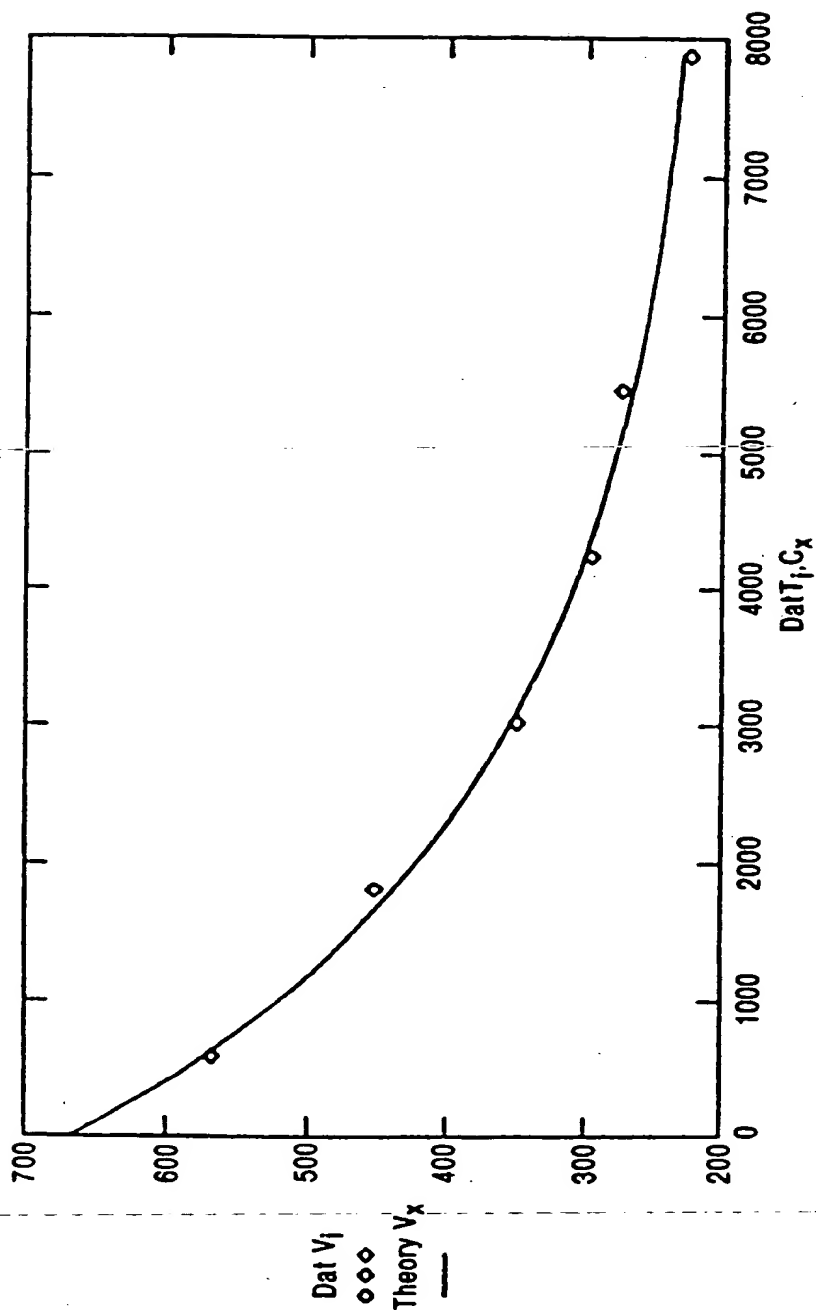


FIG. 50B

110 / 136



Representative Conc versus Time Plot. Shown is the kinetic data for 6G4V11N35A.IgG1

SAMPLE	k_a	k_d	K_d
Murine 6G4.2.5 IgG2a	8.3×10^5	2.9×10^{-4}	350pM
6G4V11N35A-IgG1	8.7×10^5	7.7×10^{-5}	88pM
6G4V11N35E-IgG1	3.0×10^6	1.4×10^{-4}	49pM

FIG. 51

111 / 136

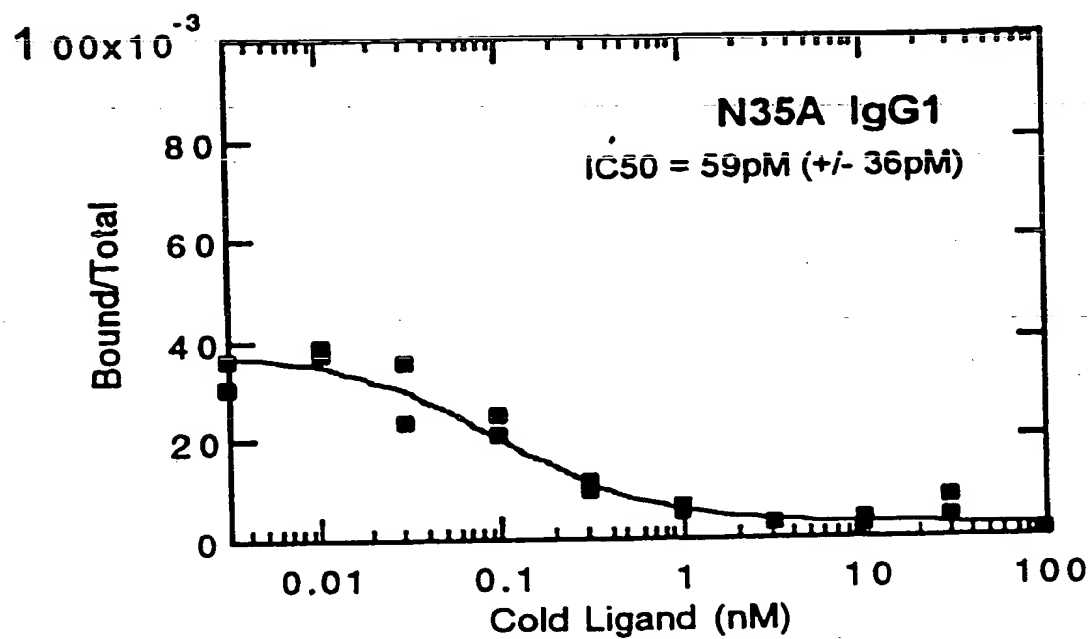


FIG. 52A

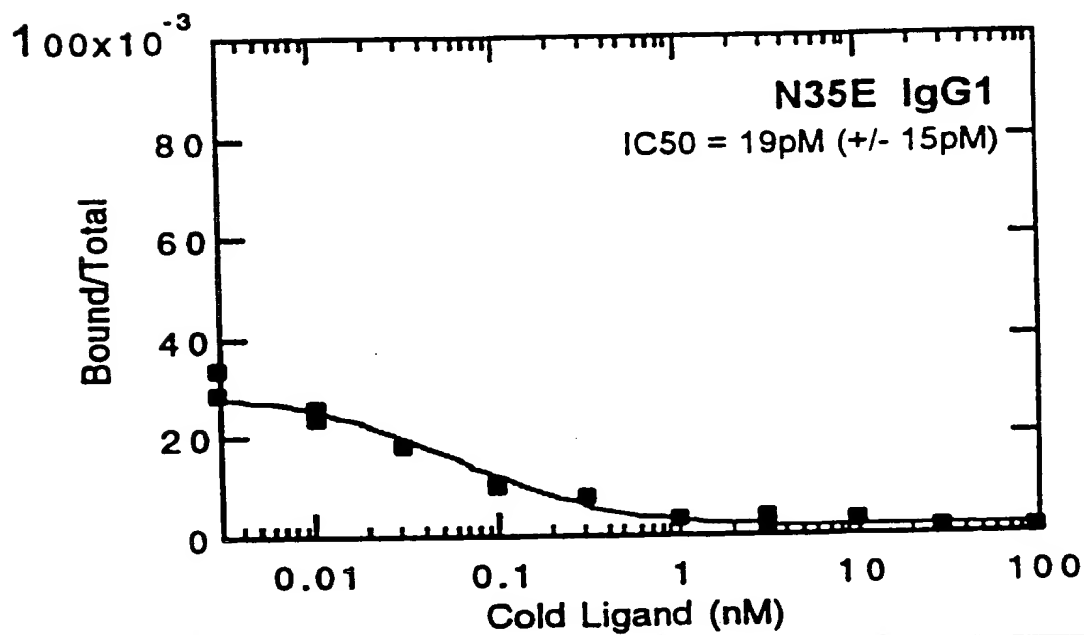


FIG. 52B

112 / 136

781 AAAAGGGTAT CTAGAGGTTG AGGTGATTTT ATGAAAAAGA ATATCGCATT TCTTCTTGCA
TTTTCCCATATA GATCTCCAAC TCCACTAAAA TACTTTTTTCT TATAGCGTAA AGAAGAACGT
-1 M K K N I A F L L A

841 TCTATGTTTCG TTTTTTCTAT TGCTACAAAC GCGTACGCTG AGGTTTCAGCT AGTGCAGTCT
AGATACAAGC AAAAAAGATA ACGATGTTTG CGCATGCGAC TCCAAGTCGA TCACGTCAGA
-11 S M F V F S I A T N A Y A E V Q L V Q S

901 GGCGGTGGCC TGGTGCAGCC AGGGGGGCTCA CTCCGTTTGT CCTGTGCAGC TTCTGGCTAC
CCGCCACCGG ACCACGTCGG TCCCCCGAGT GAGGCAAACA GGACACGTCG AAGACCGATG
8 G G G L V Q P G G S L R L S C A A S G Y

961 TCCTTCTCGA GTCACTATAT GCACTGGGTC CGTCAGGCCC CGGGTAAGGG CCTGGAATGG
AGGAAGAGCT CAGTGATATA CGTGACCCAG GCAGTCCGGG GCCCATTCCC GGACCTTACC
28 S F S S H Y M H W V R Q A P G K G L E W

1021 GTTGGATATA TTGATCCTTC CAATGGTGAA ACTACGTATA ATCAAAAGTT CAAGGGCCGT
CAACCTATAT AACTAGGAAG GTTACCACTT TGATGCATAT TAGTTTTCAA GTTCCCGGCA
48 V G Y I D P S N G E T T Y N O K F K G R

1081 TTCACTTTAT CTCGCGACAA CTCCAAAAAC ACAGCATACC TGCAGATGAA CAGCCTGCGT
AAGTGAATA GAGCGCTGTT GAGGTTTTTG TGTCGTATGG ACGTCTACTT GTCGGACGCA
68 F T L S R D N S K N T A Y L Q M N S L R

1141 GCTGAGGACA CTGCCGTCTA TTA CTGTGCA AGAGGGGATT ATCGCTACAA TGGTGA CTGG
CGACTCCTGT GACGGCAGAT AATGACACGT TCTCCCCTAA TAGCGATGTT ACCACTGACC
88 A E D T A V Y Y C A R G D Y R Y N G D W

1201 TTCTTCGACG TCTGGGGTCA AGGAACCCTG GTCACCGTCT CCTCGGCCTC CACCAAGGGC
AAGAAGCTGC AGACCCCAGT TCCTTGGGAC CAGTGGCAGA GGAGCCGGAG GTGGTTCCCG
108 F F D V W G Q G T L V T V S S A S T K G

1261 CCATCGGTCT TCCCCCTGGC ACCCTCCTCC AAGAGCACCT CTGGGGGCAC AGCGGCCCTG
GGTAGCCAGA AGGGGGACCG TGGGAGGAGG TTCTCGTGGA GACCCCCGTG TCGCCGGGAC
128 P S V F P L A P S S K S T S G G T A A L

1321 GGCTGCCTGG TCAAGGACTA CTTCCTCCGAA CCGGTGACGG TGTCGTGGAA CTCAGGCGCC
CCGACGGACC AGTTCTTGAT GAAGGGGCTT GGCCACTGCC ACAGCACCTT GAGTCCGCGG
148 G C L V K D Y F P E P V T V S W N S G A

1381 CTGACCAGCG GCGTGACAC CTTCCCGGCT GTCCTACAGT CCTCAGGACT CTACTCCCTC
GACTGGTTCG CGCACGTGTG GAAGGGCCGA CAGGATGTCA GGAGTCCTGA GATGAGGGAG
168 L T S G V H T F P A V L Q S S G L Y S L

1441 AGCAGCGTGG TGACCGTGCC CTCCAGCAGC TTGGGCACCC AGACCTACAT CTGCAACGTG
TCGTGCACACC ACTGGCACGG GAGGTCGTG AACCCTGCG TCTGGATGTA GACGTTGCAC
188 S S V V T V P S S S L G T Q T Y I C N V

1501 AATCACAAGC CCAGCAACAC CAAGGTCGAC AAGAAAGTTG AGCCCAAATC TTGTGACAAA
TTAGTGTTCG GGTGCTGTG GTTCCAGCTG TTCTTTCAAC TCGGGTTTAG AACACTGTTT
208 N H K P S N T K V D K K V E P K S C D K

1561 ACTCACACAT GCCCGCCGTGA
TGAGTGTGTA CGGGCCGGCACT
228 T H T C P P O

FIG. 53

113 / 136

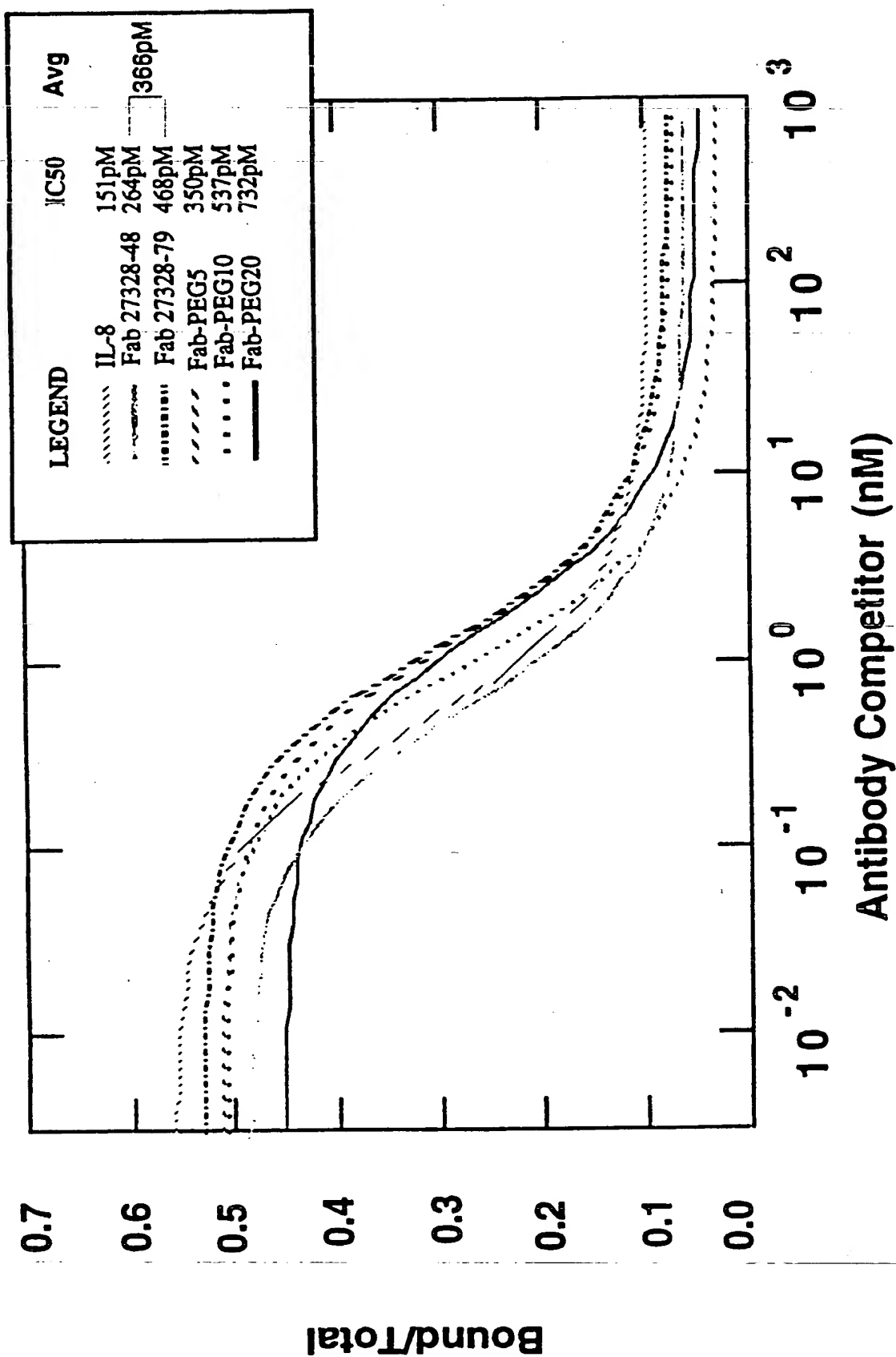


FIG. 54A

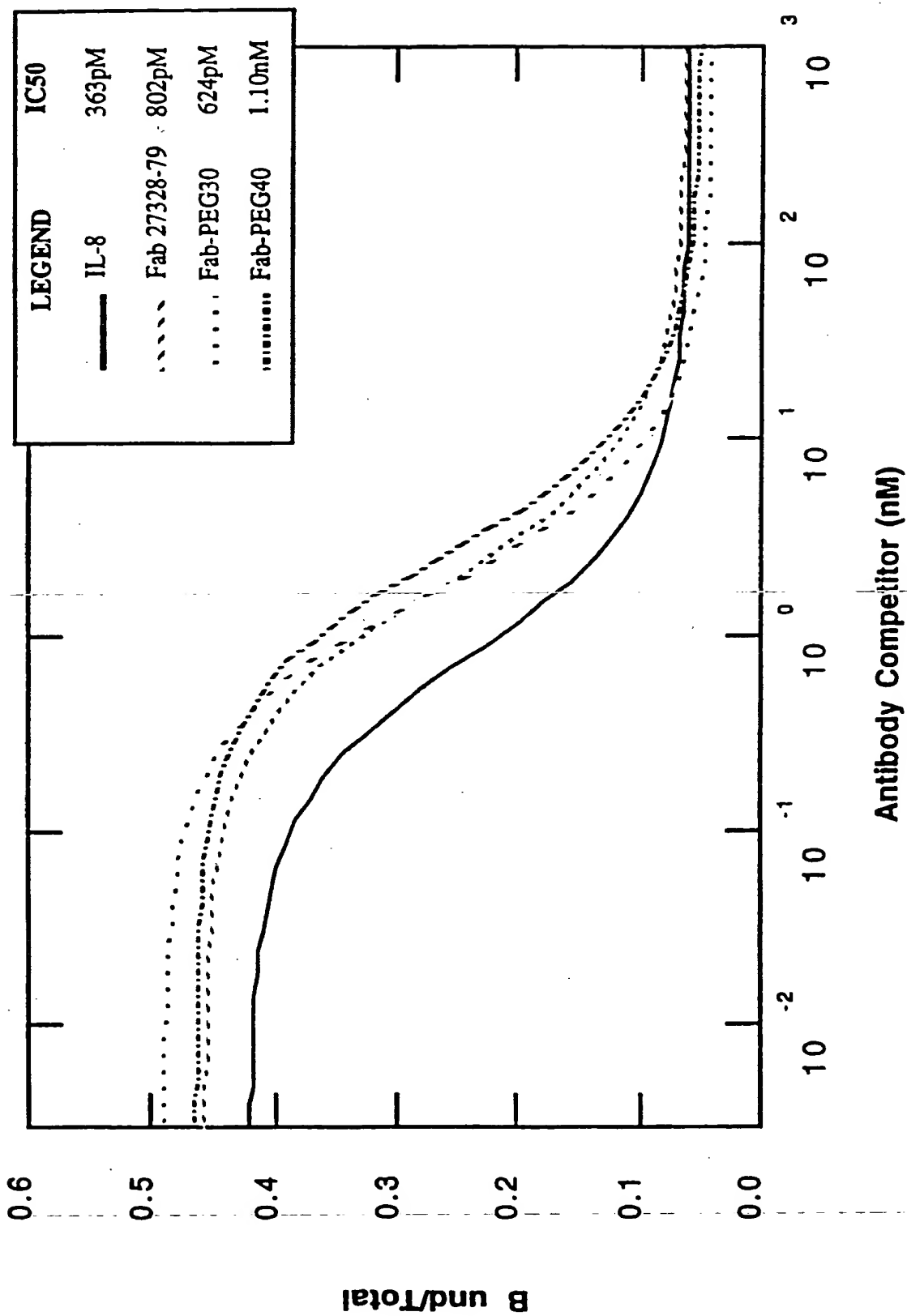
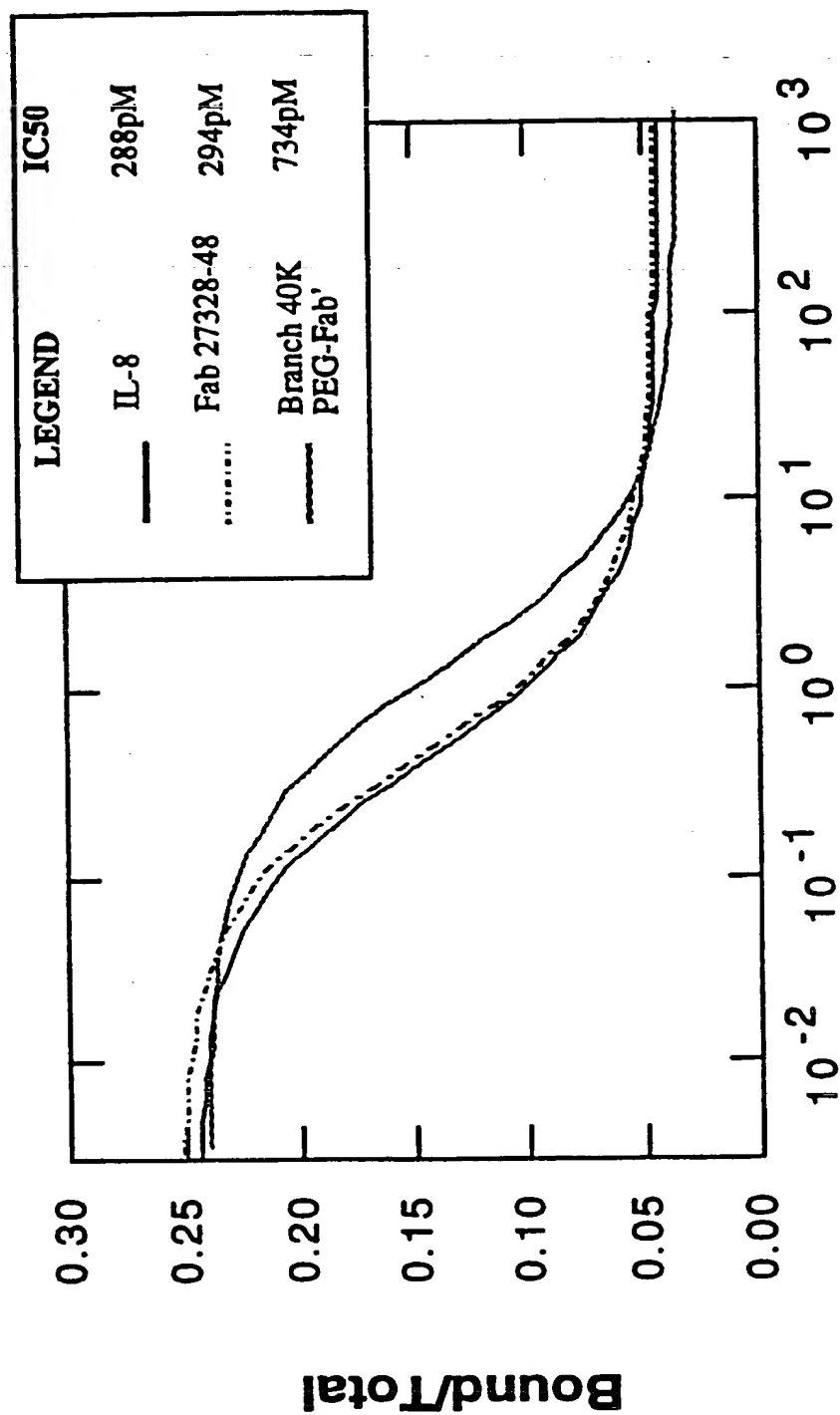


FIG. 54B

115 / 136



Antibody Competitor (nM)

FIG. 54C

116 / 136

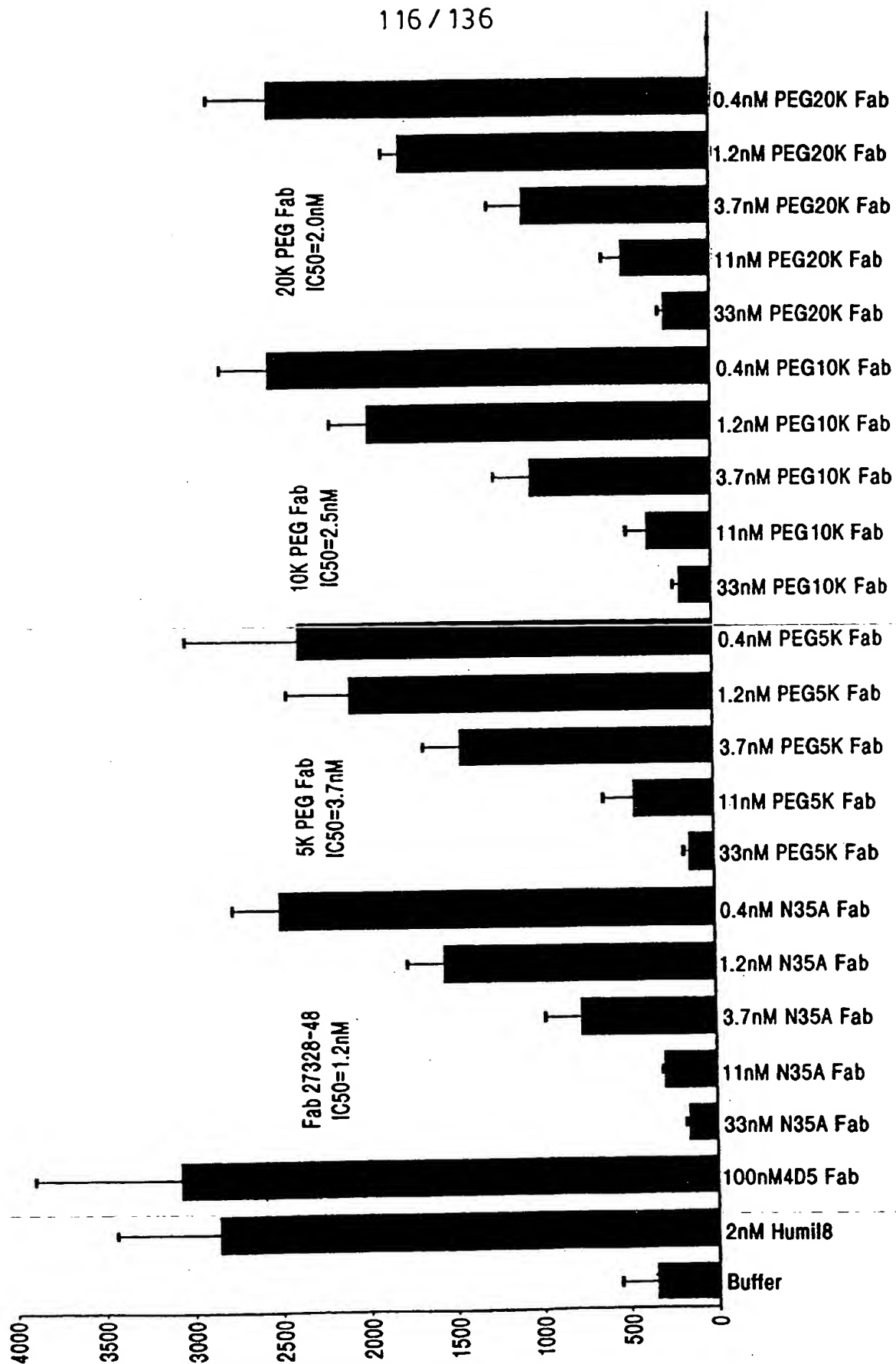


FIG. 55A

117 / 136

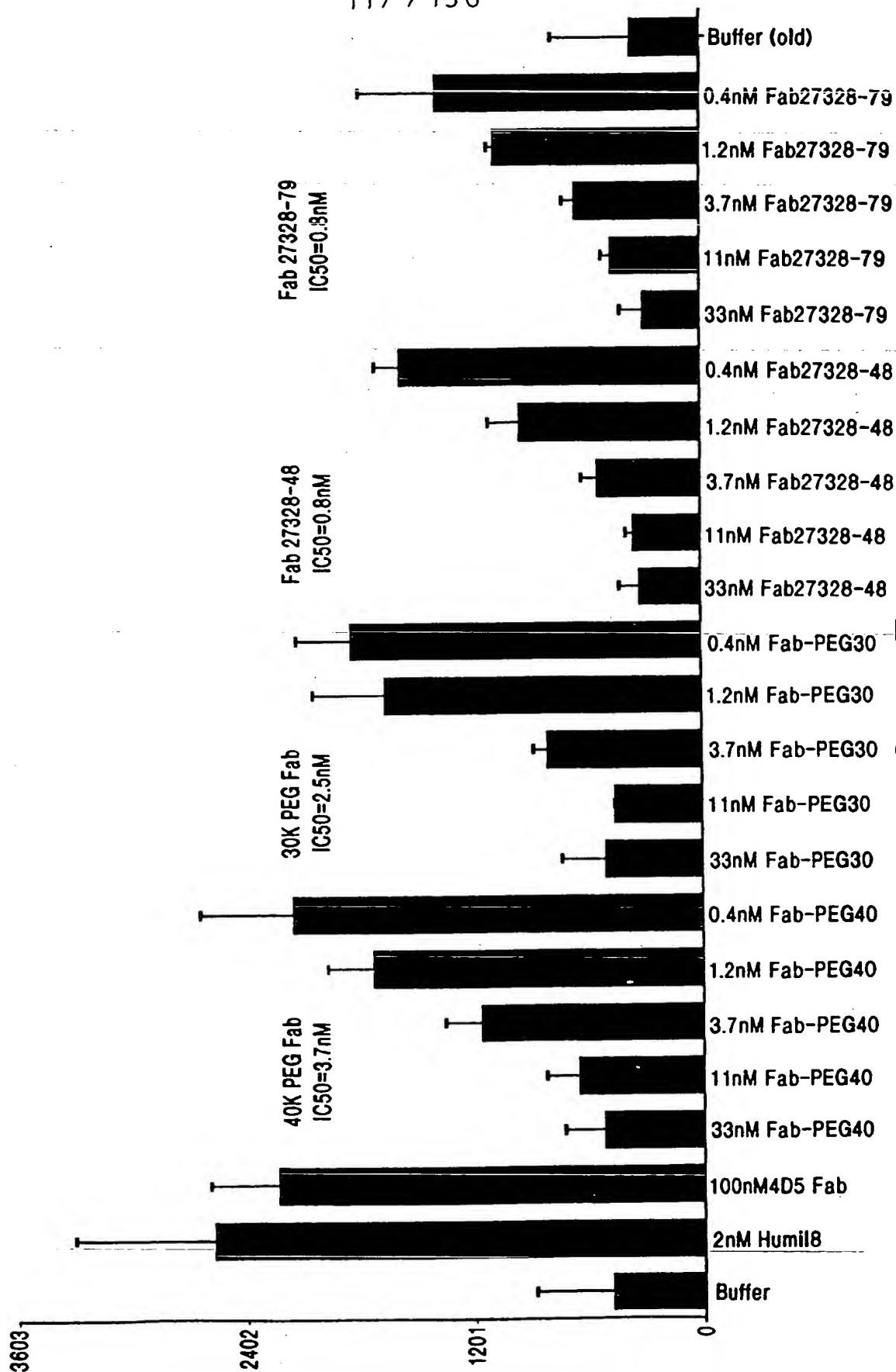


FIG. 55B

118 / 136

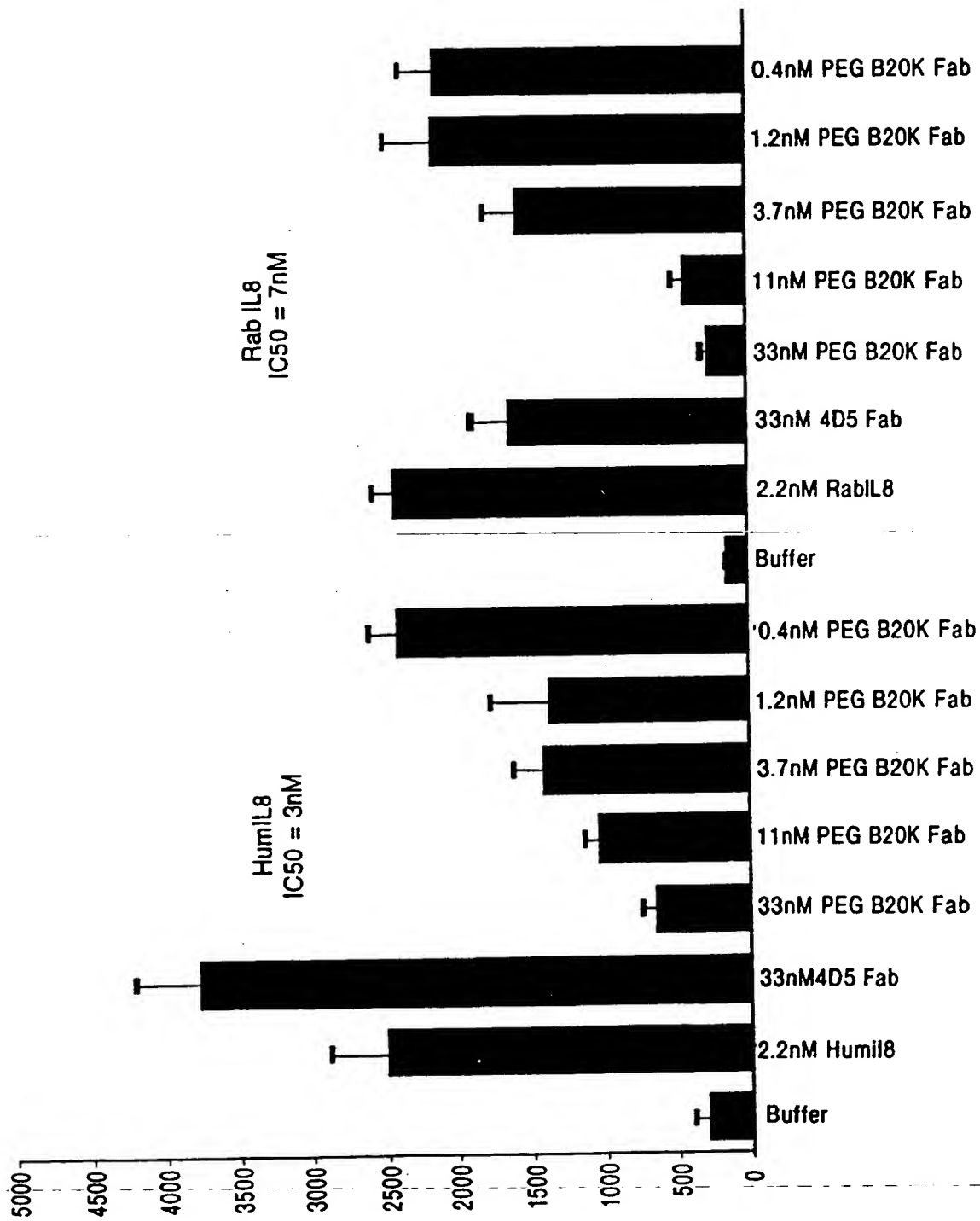
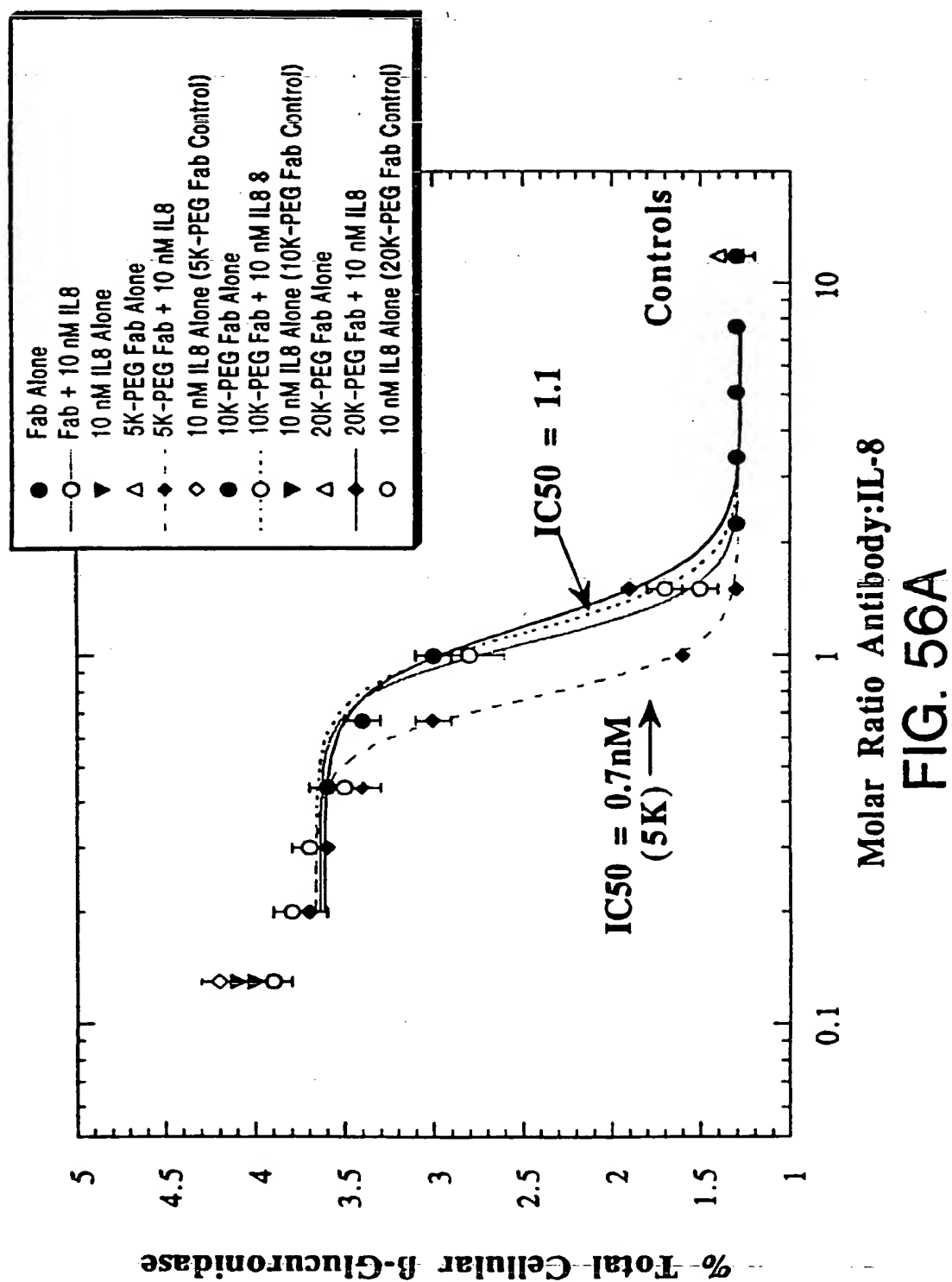


FIG. 55C

119 / 136



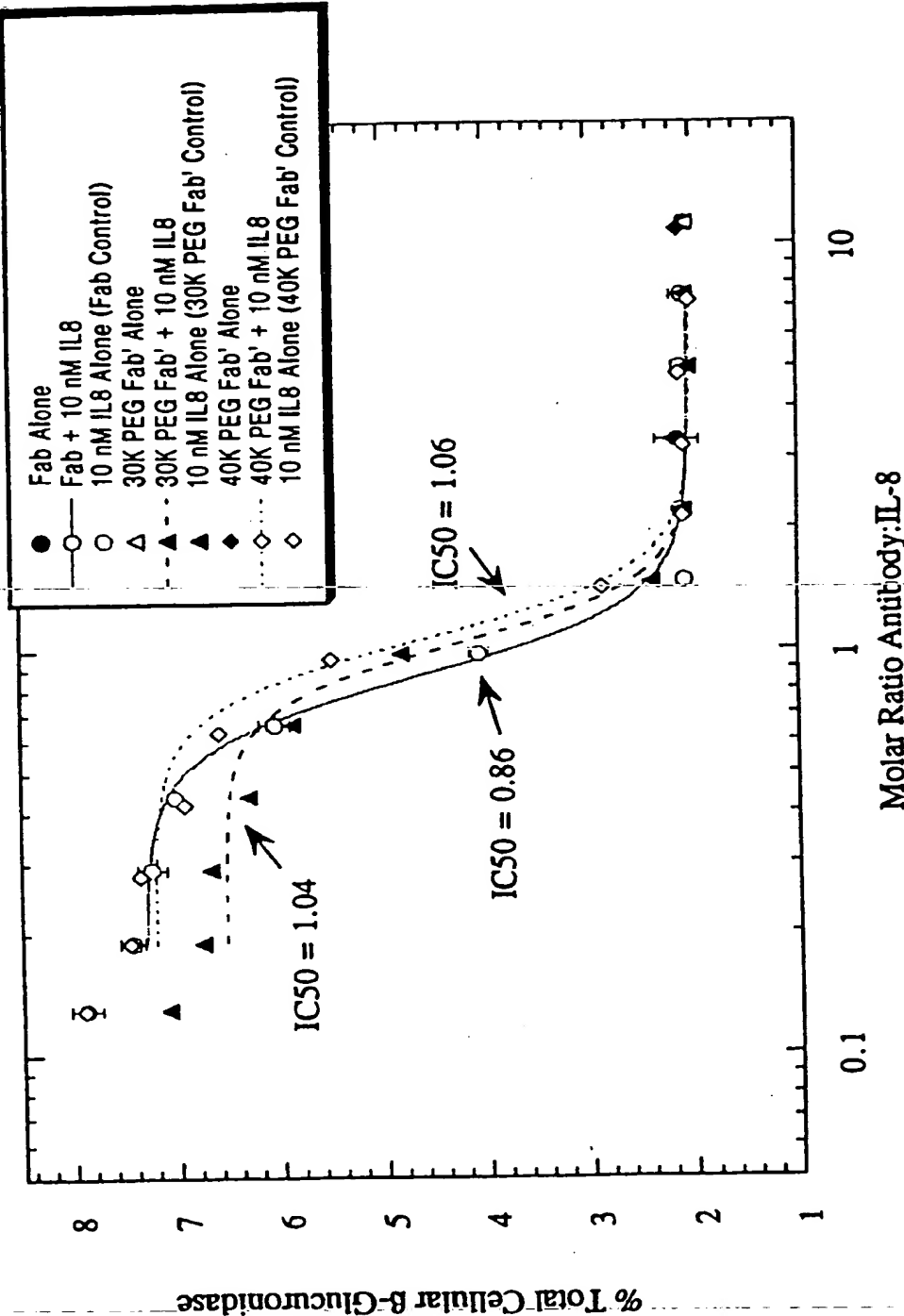
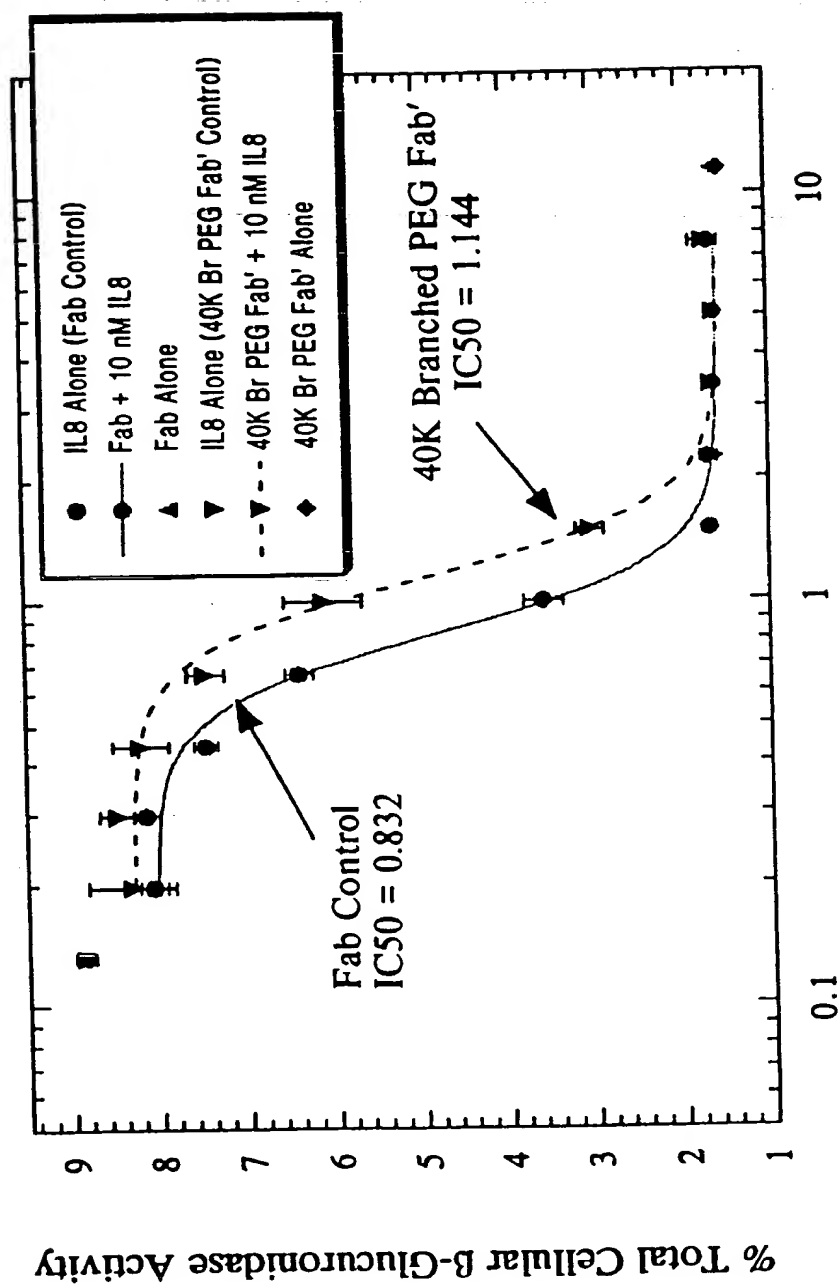


FIG. 56B

121 / 136



Molar Ratio Antibody:IL8

FIG. 56C

122/136

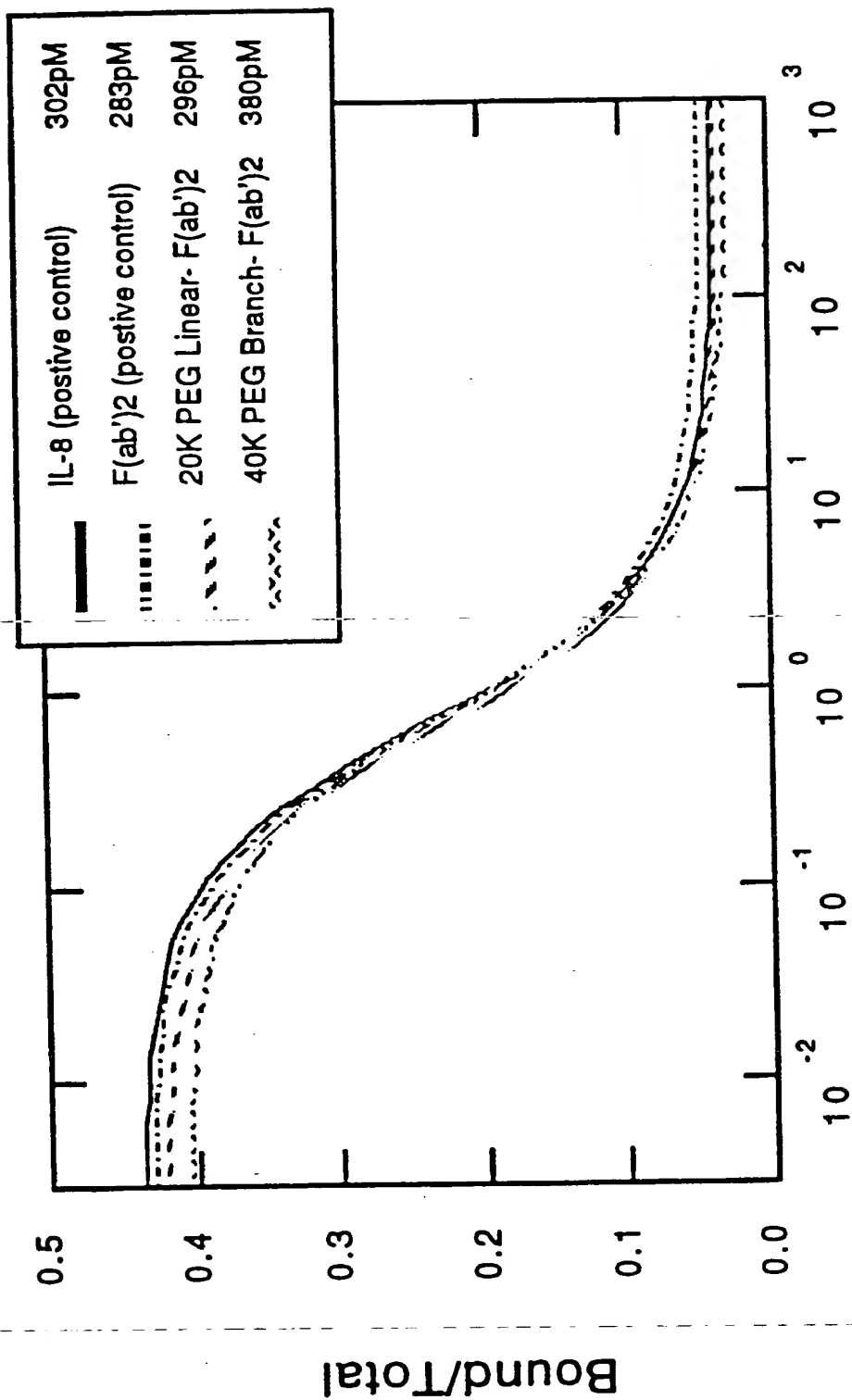
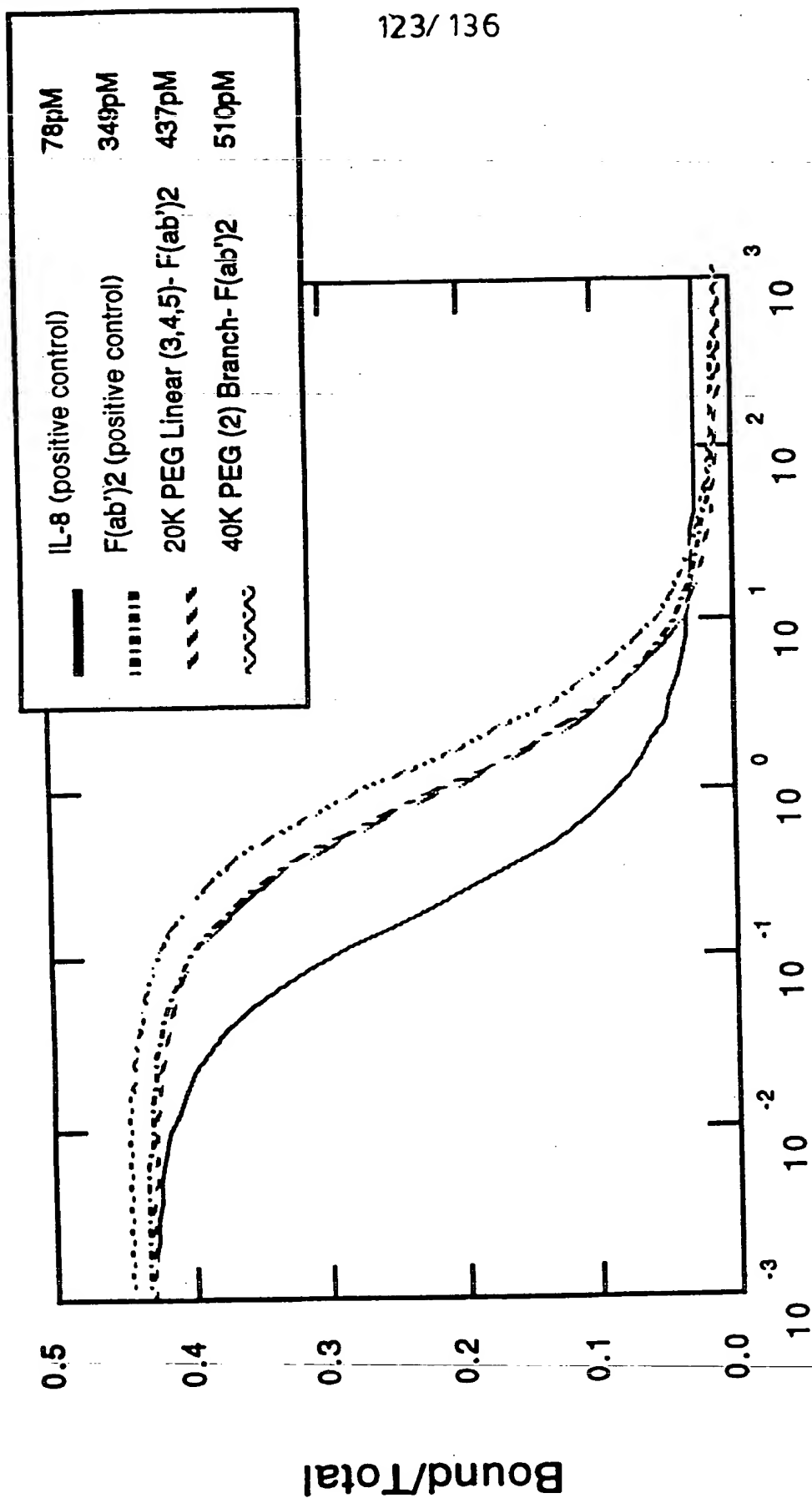
Pegylated F(ab')₂ (nM)

FIG. 57A

123/ 136



Pegylated F(ab')₂ (nM)

FIG. 57B

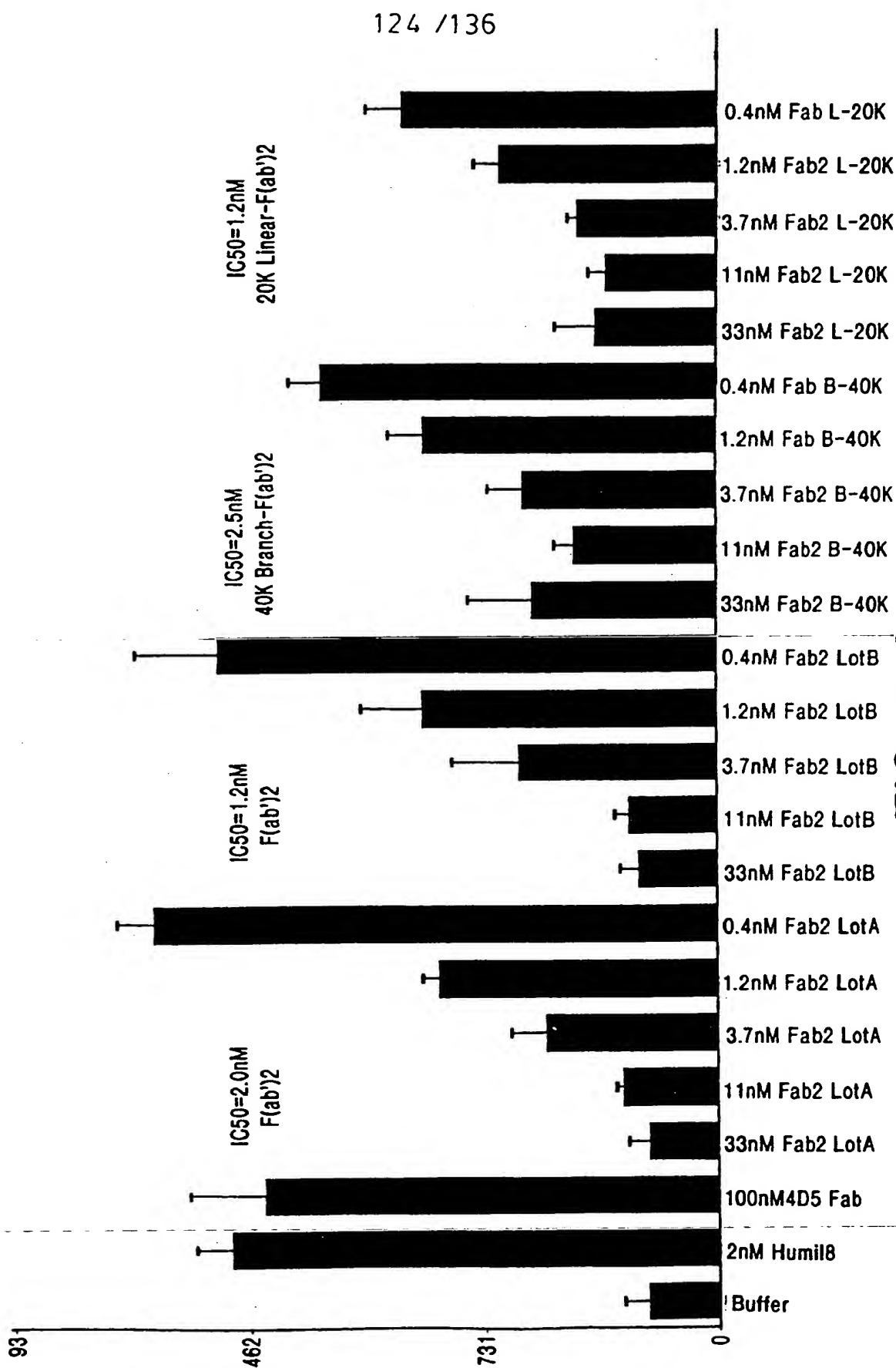


FIG. 58A

125 / 136

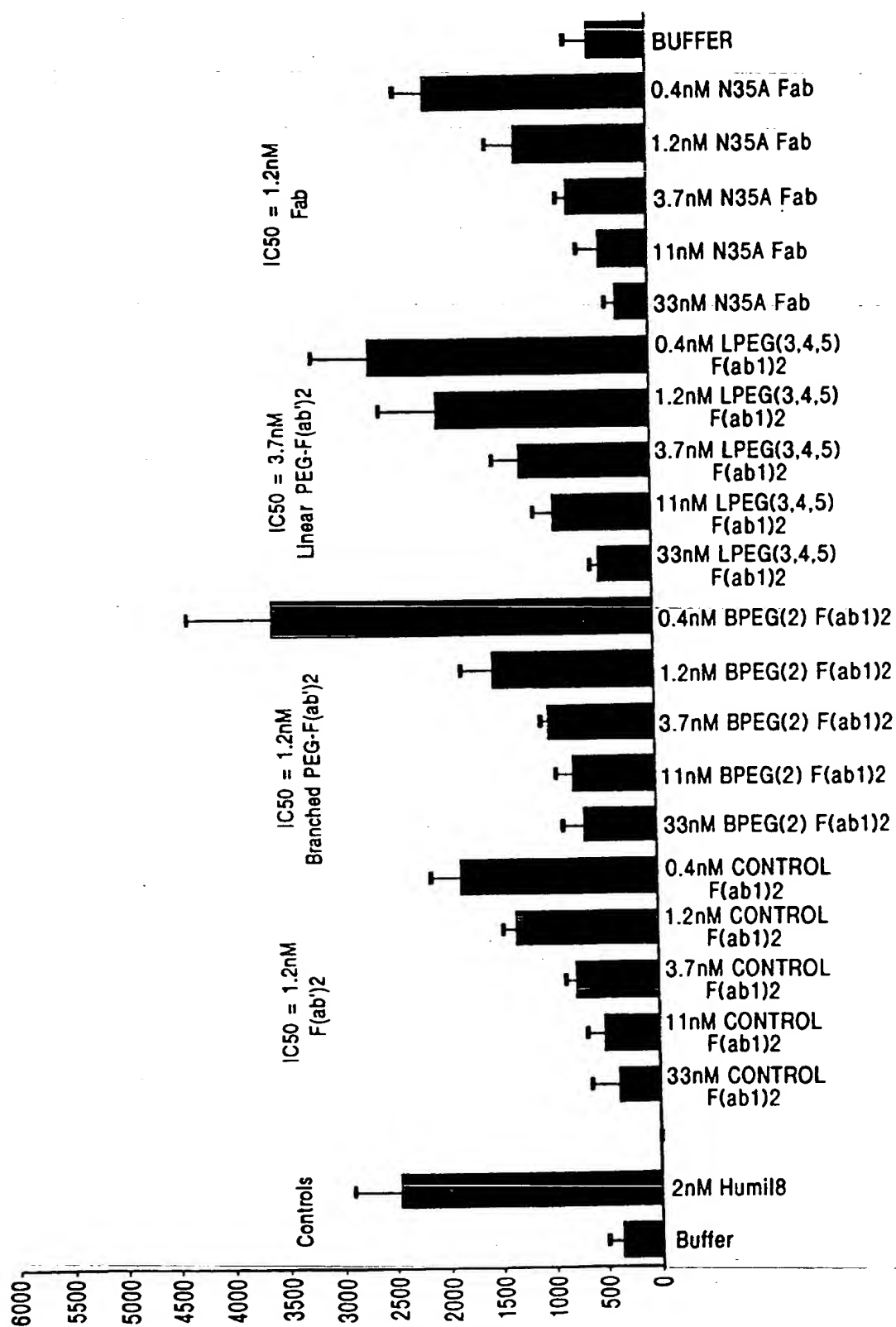


FIG. 58B

126 / 136

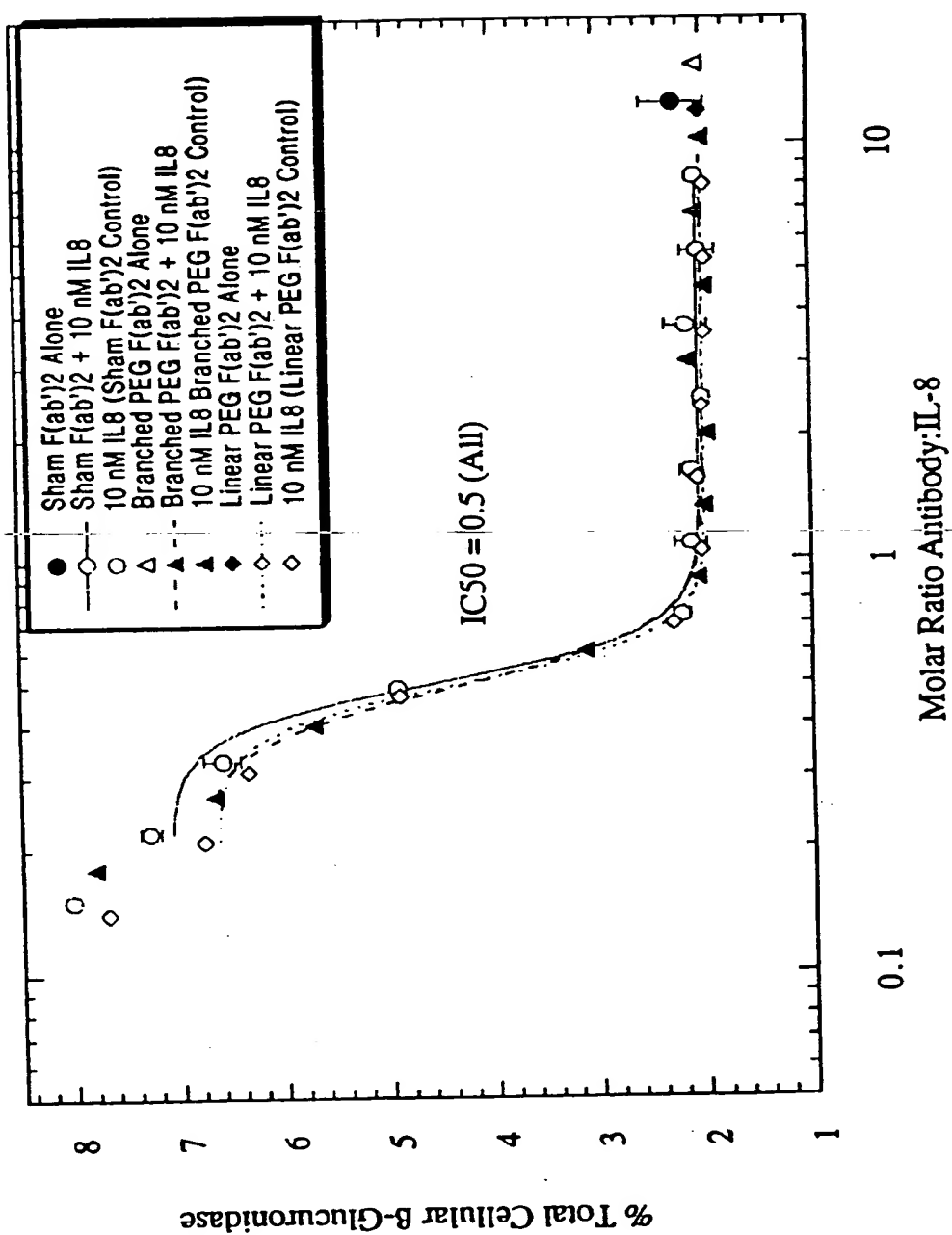


FIG. 59A

127 / 136

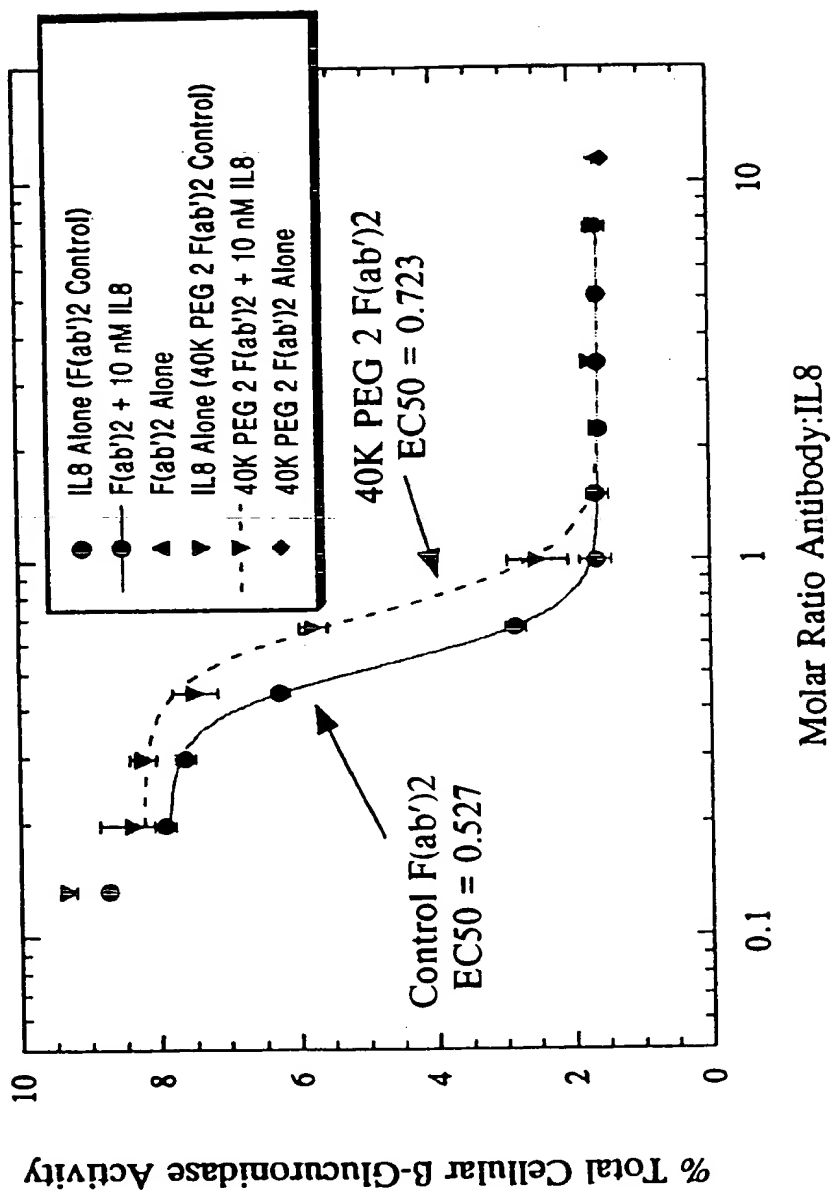


FIG. 59B

128/136

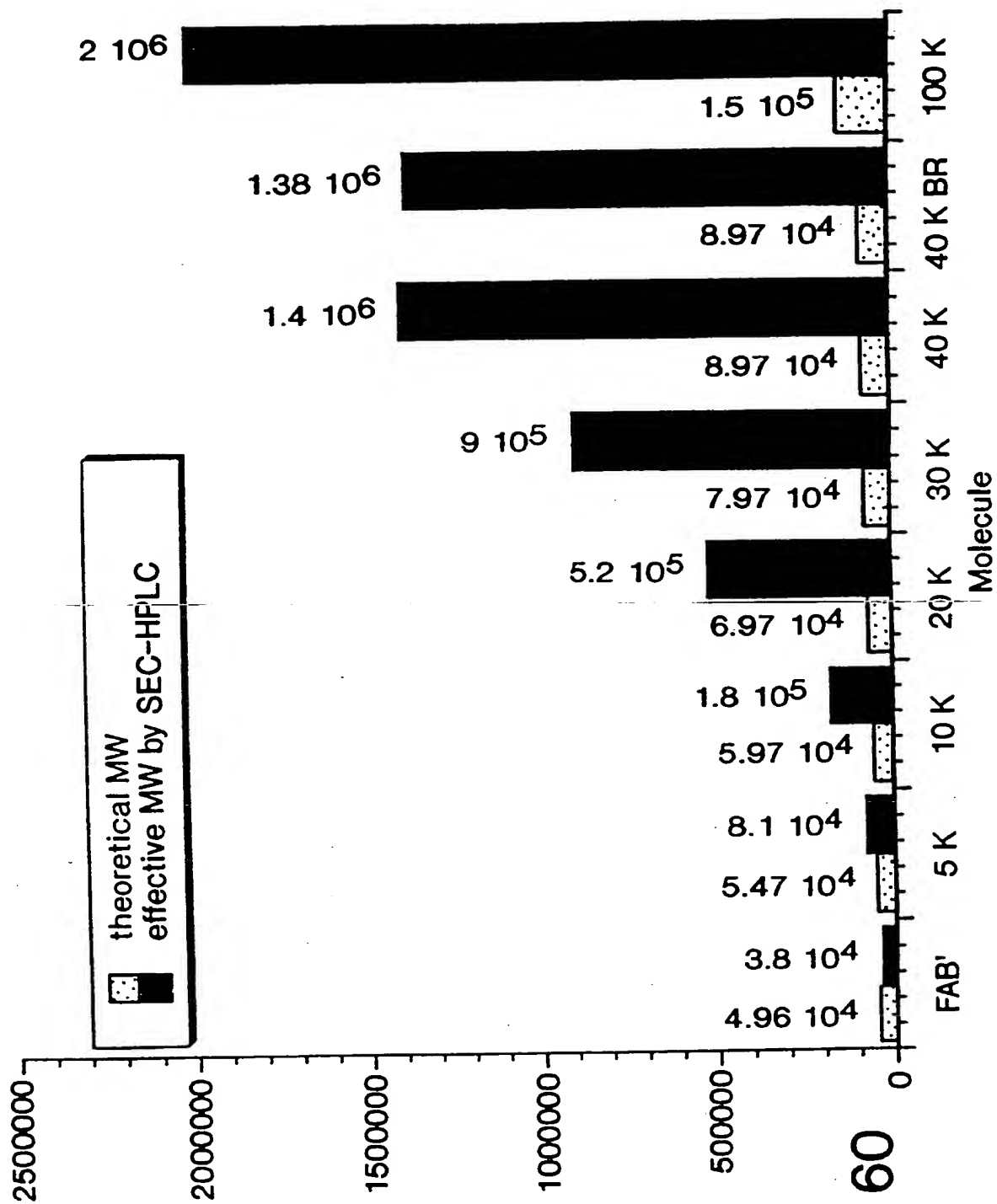
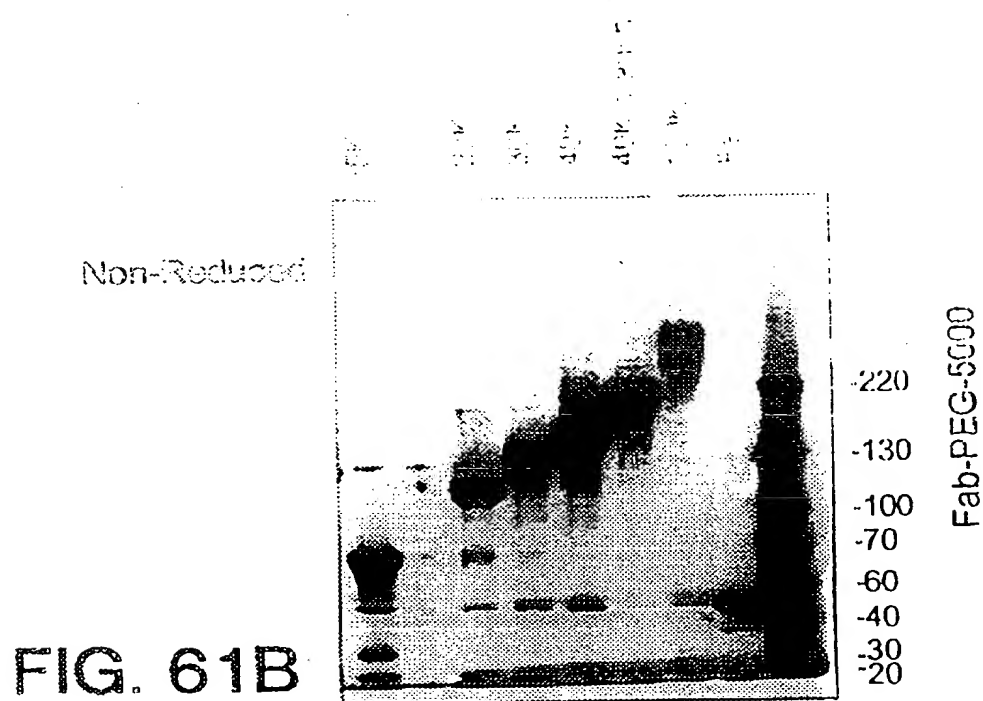
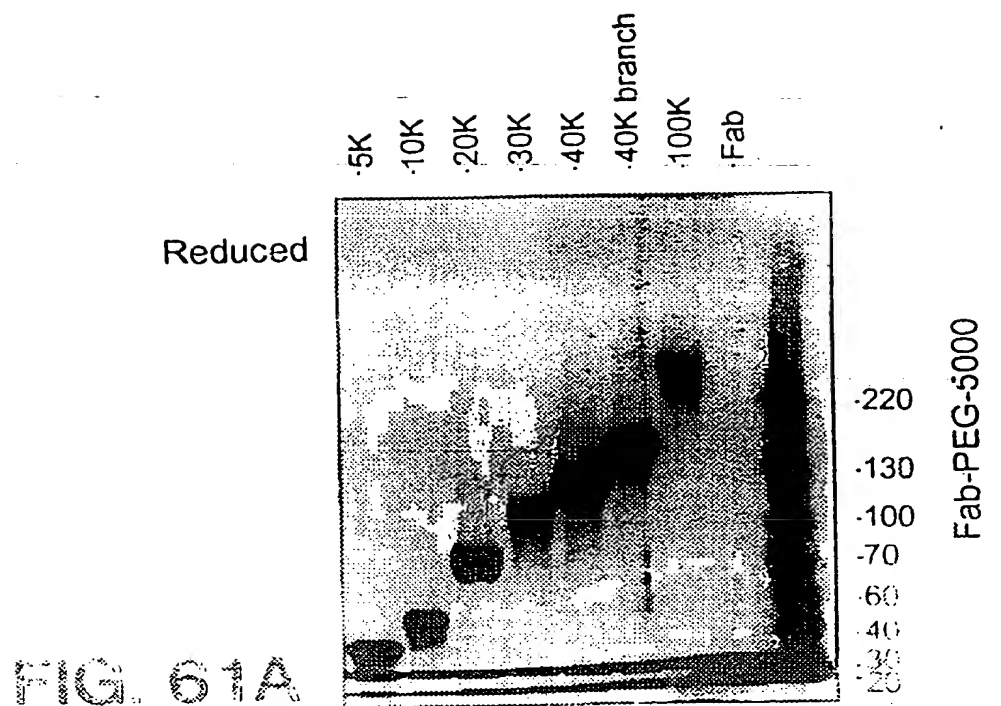


FIG. 60

(D) MW

129 / 136



130 / 136

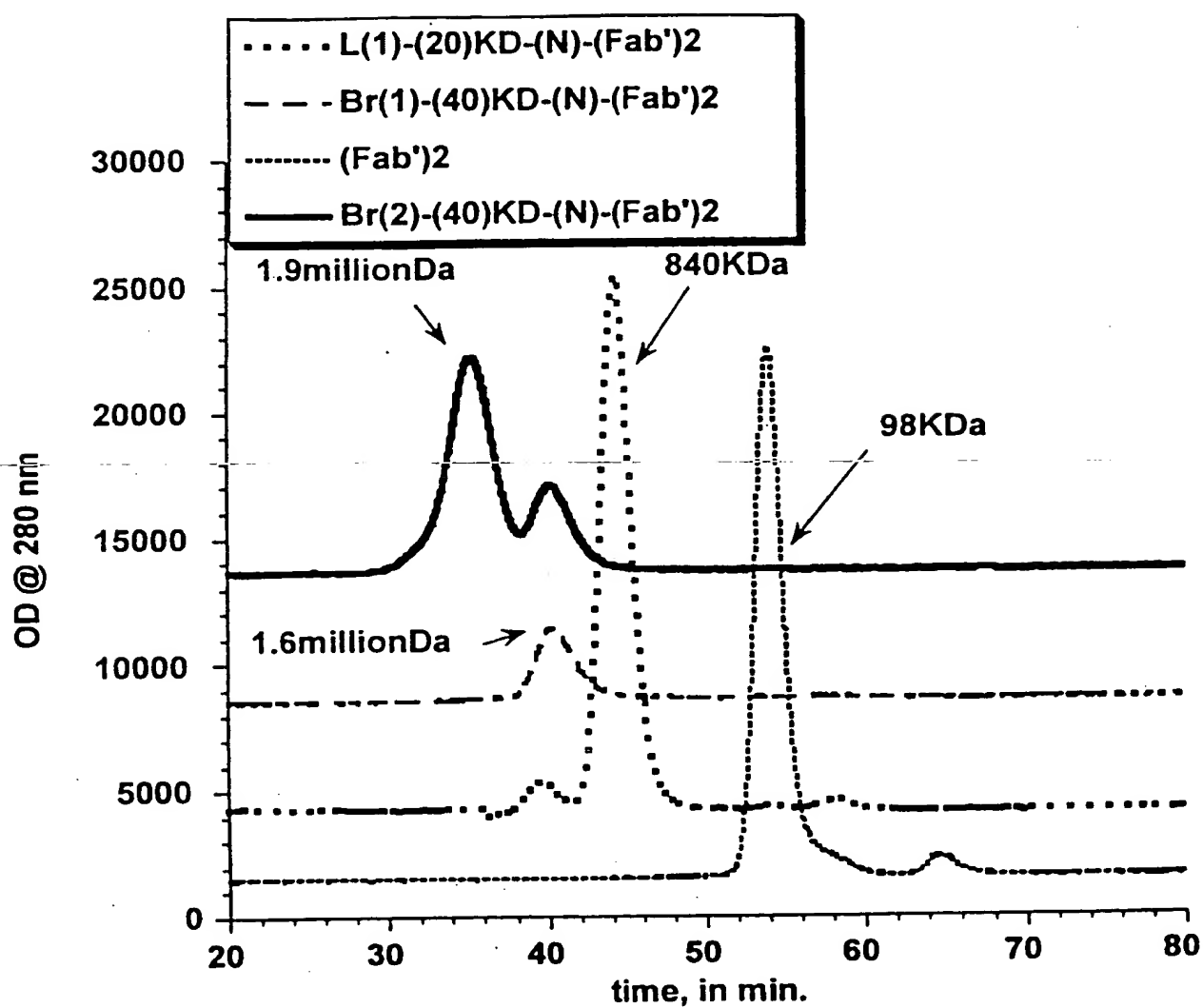


FIG. 62

131 / 136

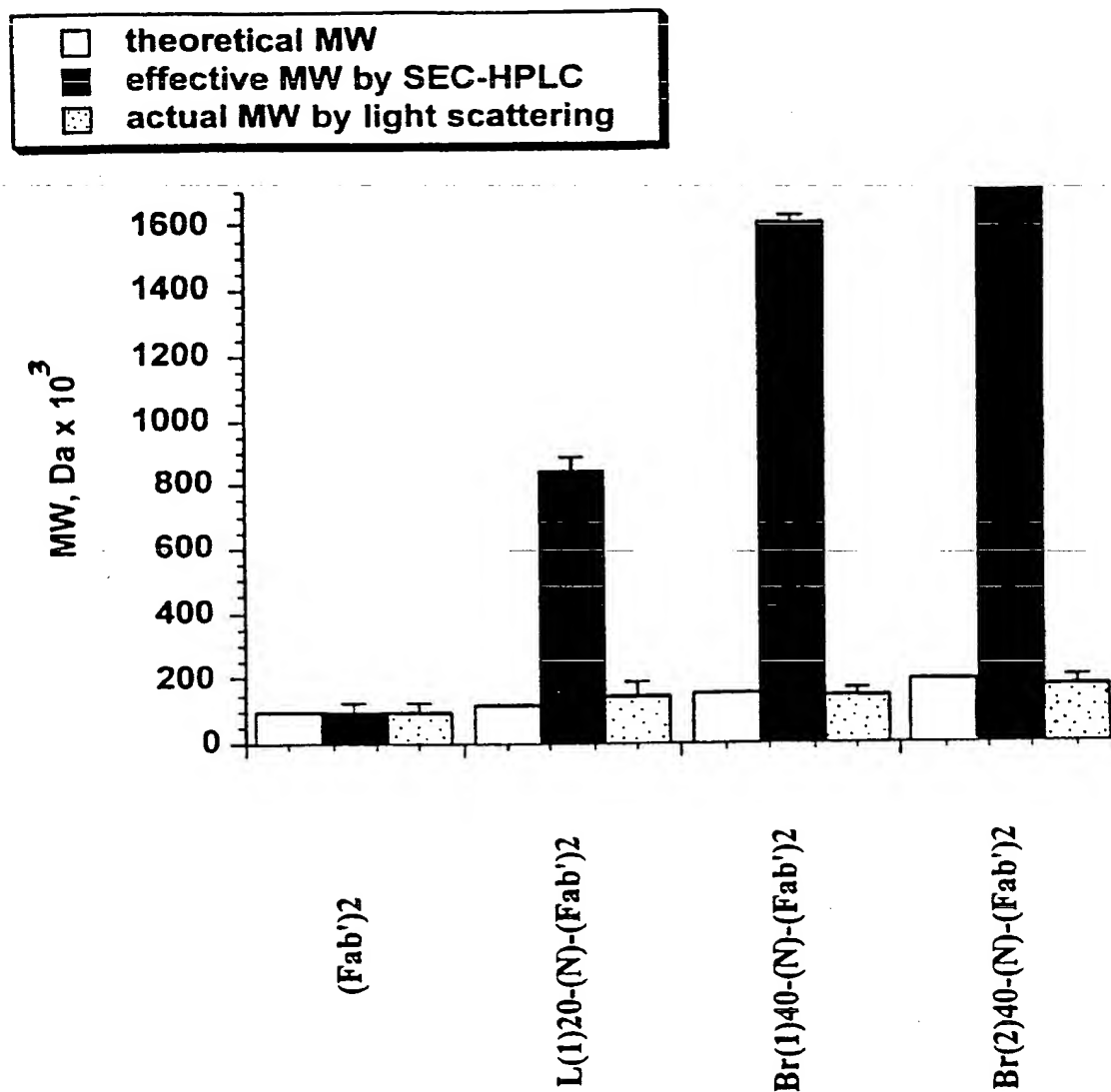


FIG. 63

13 2 / 136

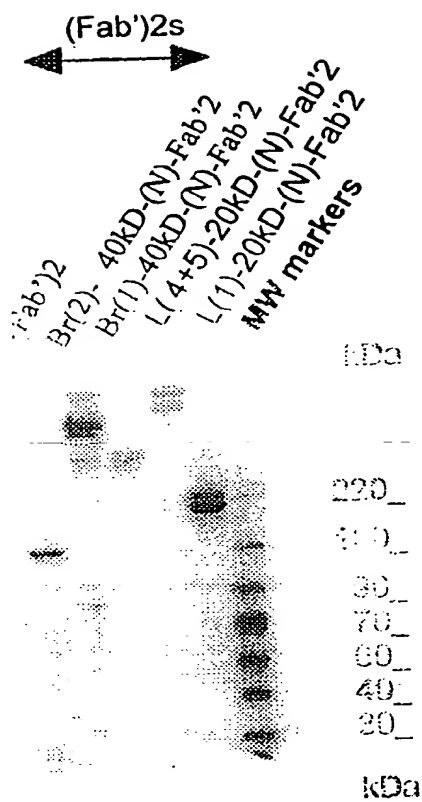
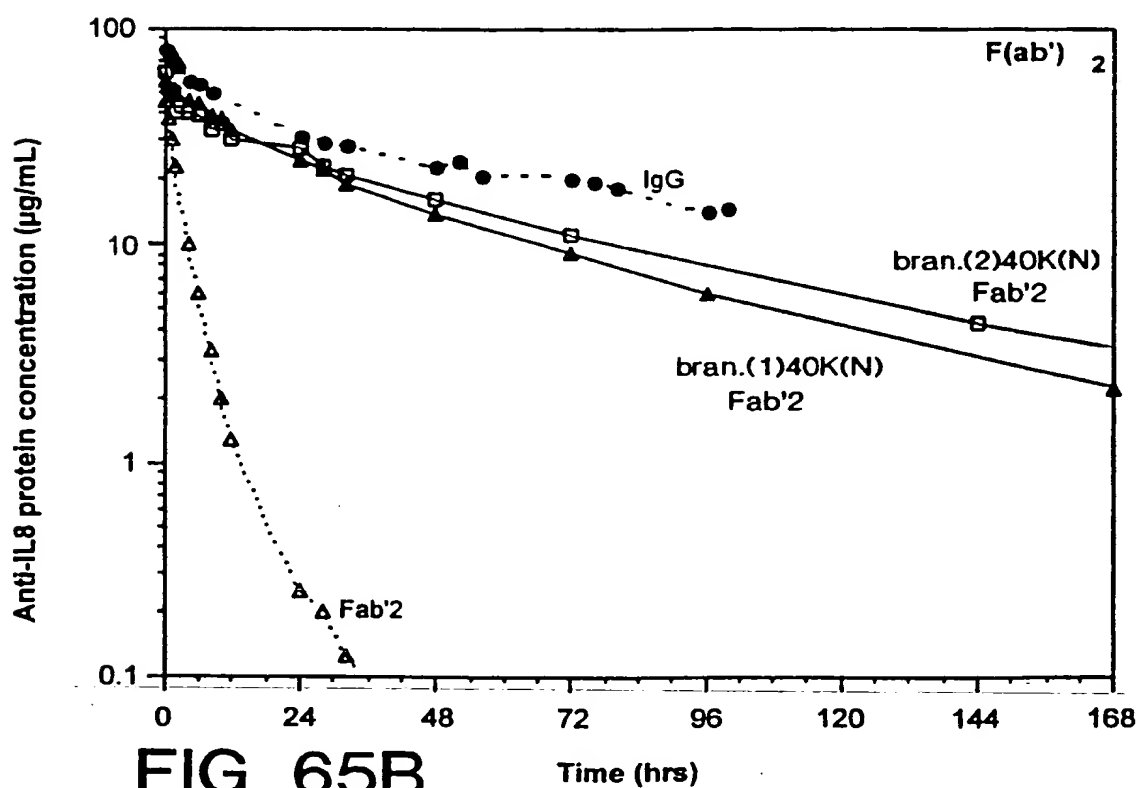
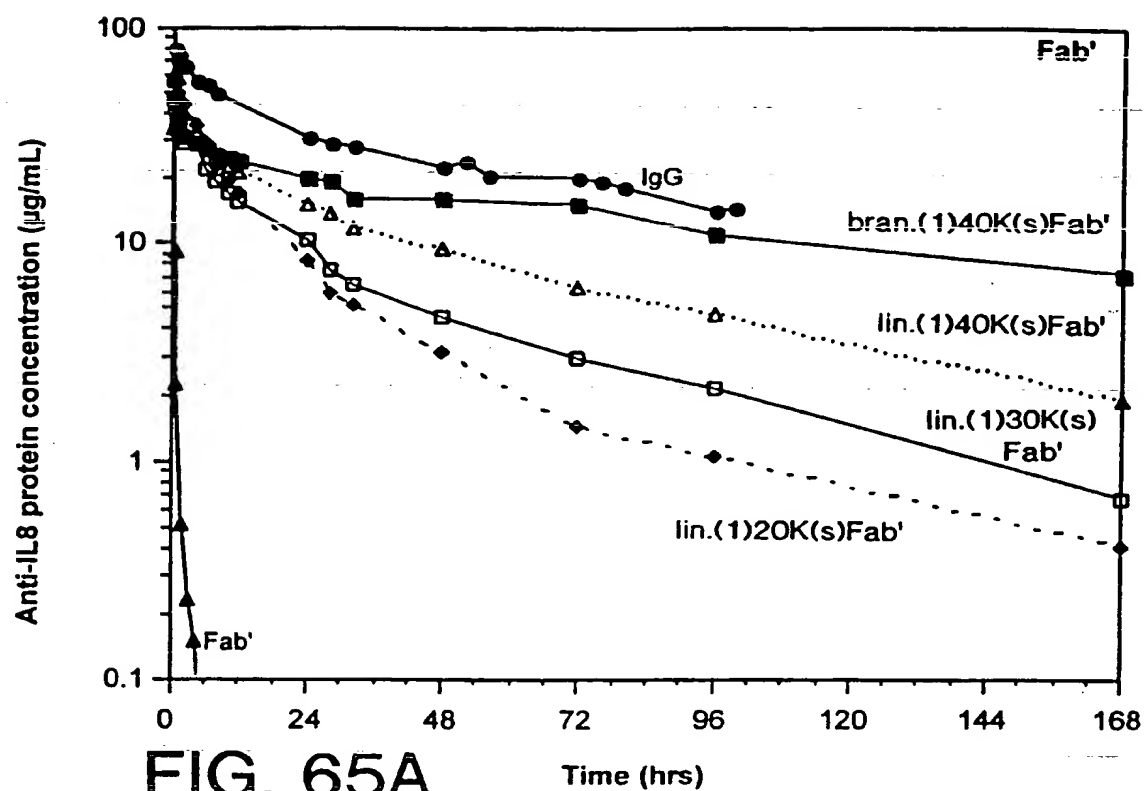


FIG. 64

133 / 136



134/136

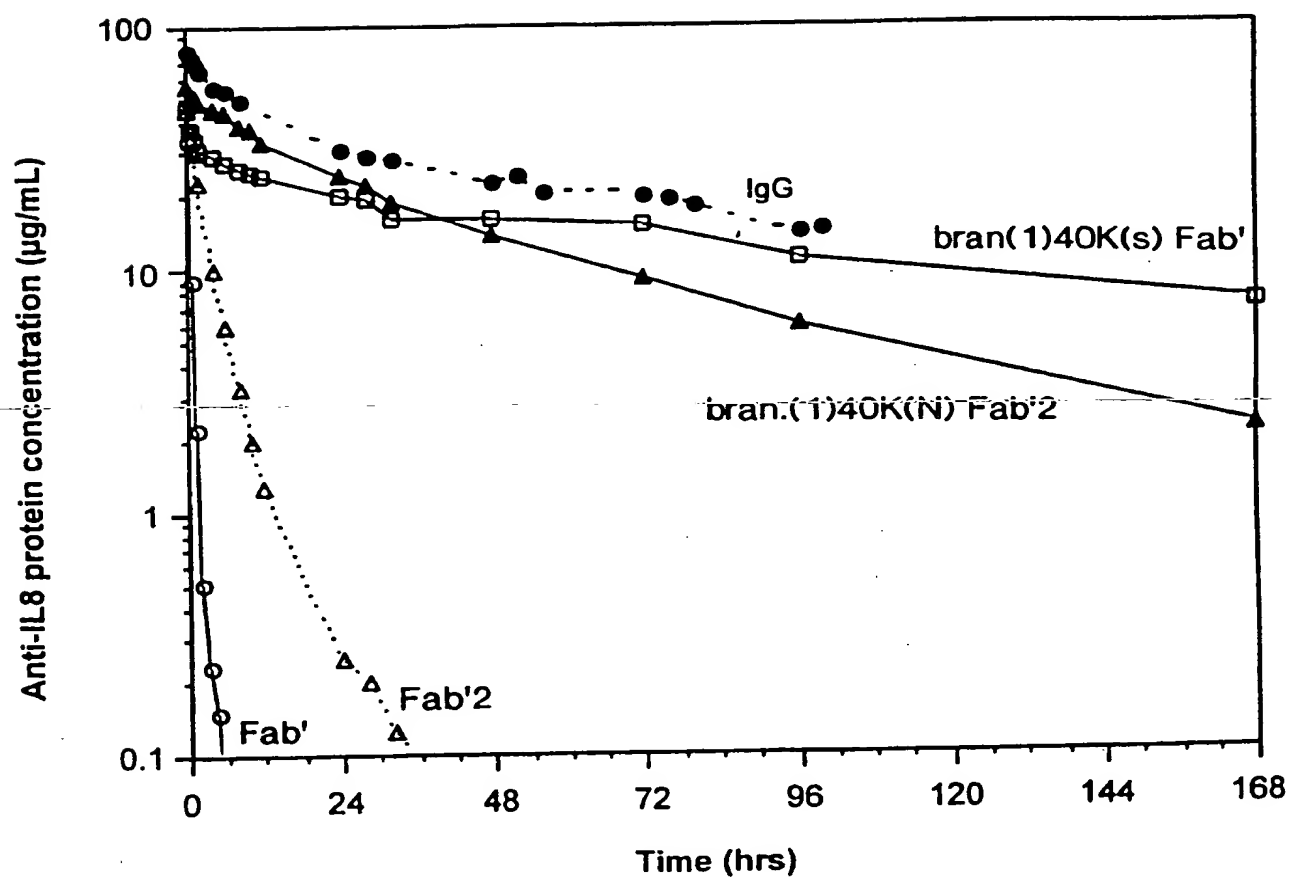


FIG. 66

135/136

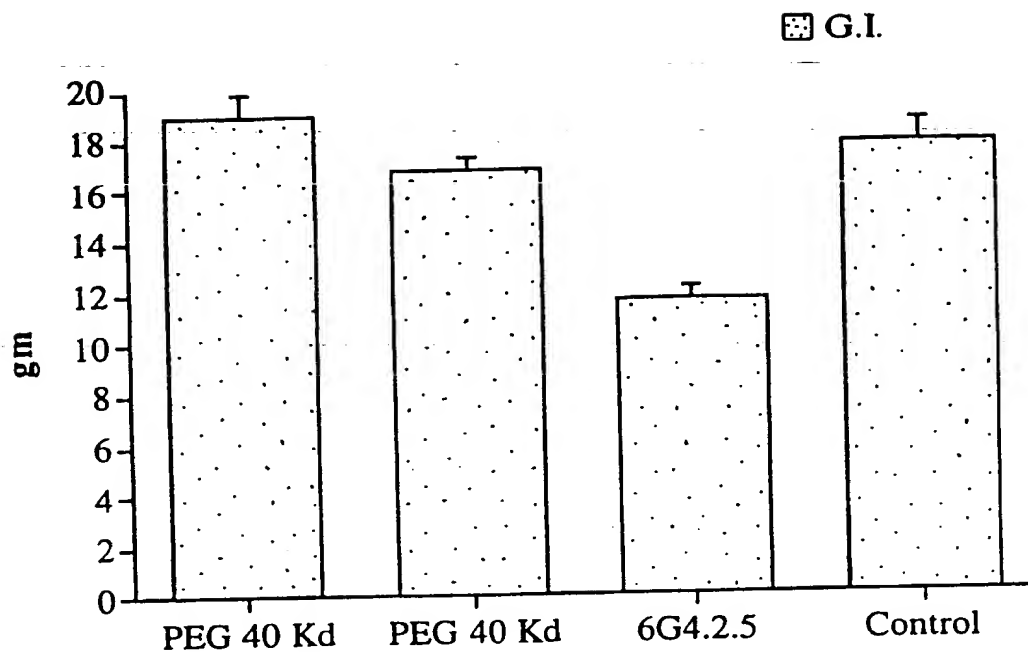


FIG. 67

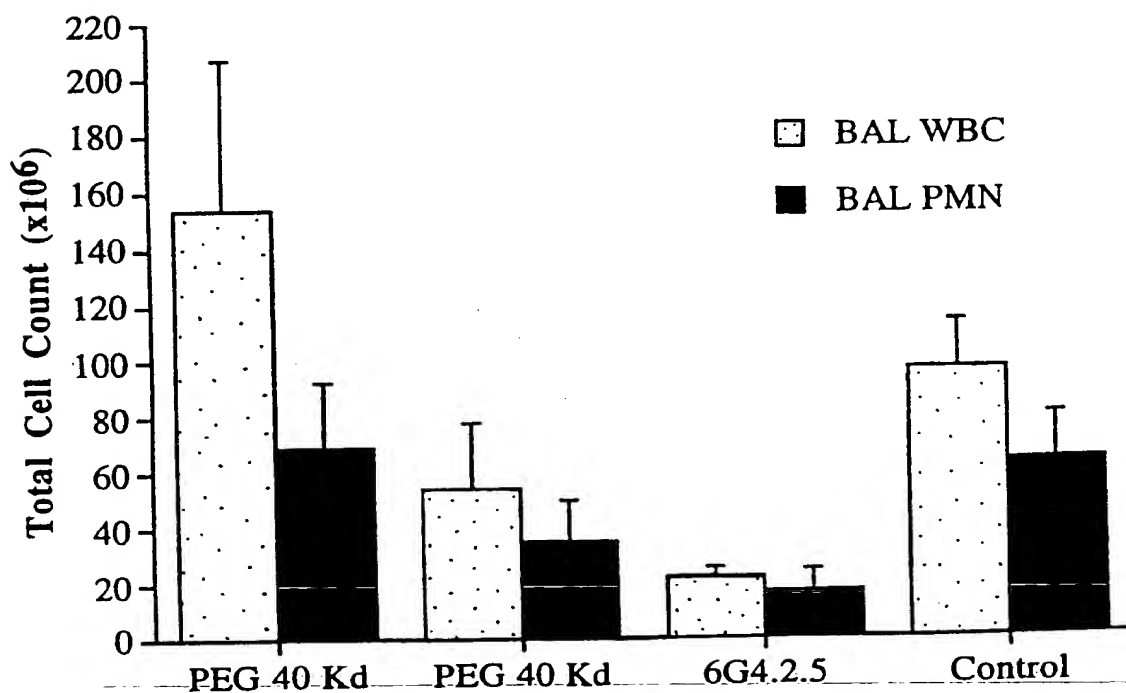


FIG. 68

136 / 136

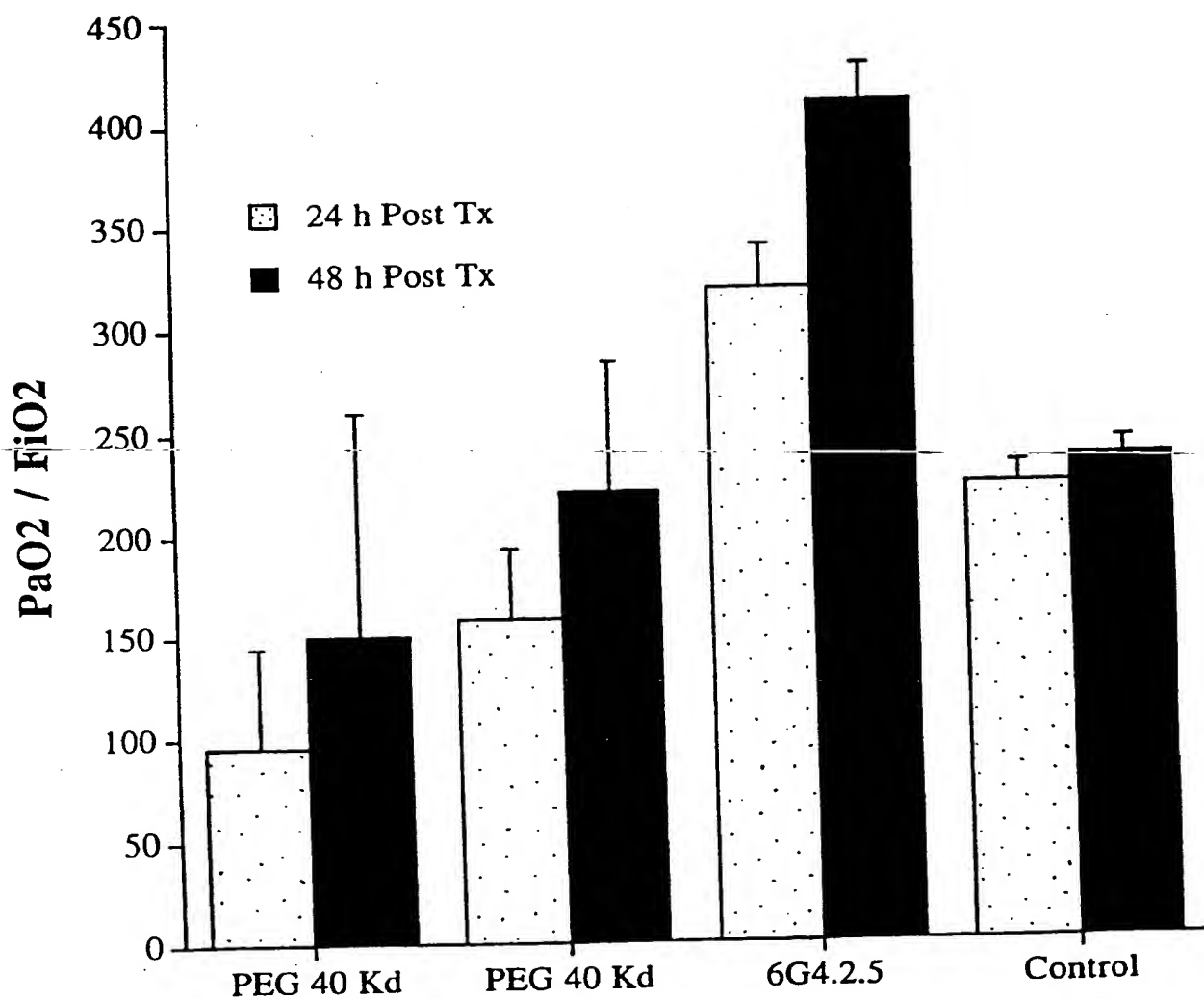


FIG. 69